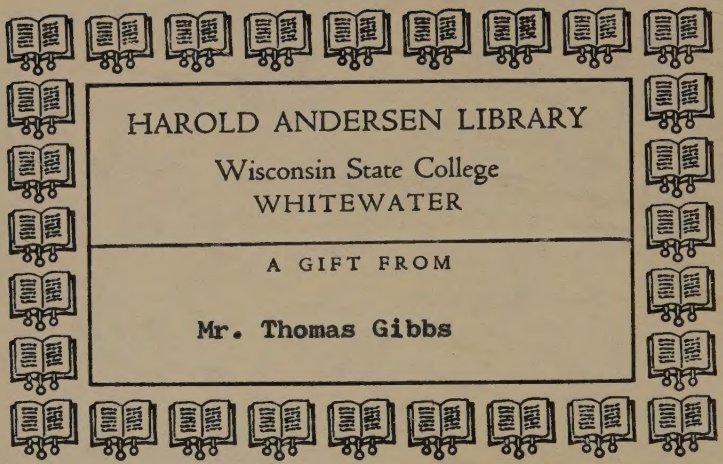

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AGRICULTURAL AND INDUSTRIAL BACTERIOLOGY

AGRICULTURAL AND INDUSTRIAL BACTERIOLOGY

BY

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PREFACE

AGRICULTURAL AND INDUSTRIAL BACTERIOLOGY is based on practical experience as a teacher and lecturer during the last sixteen years, and has been written to supply a need constantly felt during these years of practical work with students of agriculture—the need for a book to include those bacteriological topics fundamental in everyday life on the farm and in the industries. The volume has also been written for textbook use and to serve as a reference work for students and the many men and women interested in agricultural and industrial problems.

Written along such lines, and for such a public, AGRICULTURAL AND INDUSTRIAL BACTERIOLOGY contains no detailed discussion of methods and technique, since it is not intended to serve as a manual of laboratory practice. This volume has been written to lay a satisfactory and practical foundation on which may be based more advanced work in the specialized applications of bacteriology in dairying, soil technology, forestry, plant pathology, and various industries such as bottling, baking and canning.

In certain parts of the book it is assumed that the reader possesses some knowledge of chemistry and biology; but the major portion of the volume requires no such background of previous work and preparation.

R. E. BUCHANAN.

IOWA STATE COLLEGE.

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SECTION I

MORPHOLOGY AND CLASSIFICATIONS OF ORGANISMS

AGRICULTURAL AND INDUSTRIAL BACTERIOLOGY

CHAPTER I

THE DEVELOPMENT OF AGRICULTURAL AND INDUSTRIAL BACTERIOLOGY

Bacteriology is frequently defined as that branch of science which has to do with bacteria, but in a broader sense it may include consideration likewise of those yeasts, molds, and protozoa which may be studied most conveniently and satisfactorily by the methods used in the study of the true bacteria. Those who use the term *bacteriology* in the narrower sense have adopted *microbiology* to designate that science which includes consideration of all forms of microscopic life. In this volume the term *bacteriology* will be used in the broader sense. The terms *microbe, microörganism,* and *germ* are used popularly to designate any one of these minute forms of life to be considered. Of these terms, microörganism is probably most commonly used, and is to be preferred. *Agricultural* and *industrial bacteriology* may be defined, therefore, as that branch of science which deals with microörganisms, that is, bacteria, yeasts, molds and the protozoa, as they may affect agriculture or the industries directly or indirectly.

Agricultural bacteriology draws its material from many sources. It is necessary briefly to consider the size, shape, and structure of microörganisms, that is to study *morphological bacteriology.* The relationship of bacteria and

other microörganisms to each other must be considered from the standpoint of *systematic bacteriology*. The relationships between microörganisms and their environment, such as the effect of heat and light upon their growth and activities, and the manner in which microörganisms themselves bring about changes must also be considered. This is the branch of science which may be termed *physiological bacteriology*. The agriculturist is interested in microörganisms present in foods, particularly in milk, and the changes which are brought about in dairy products, that is, in *dairy bacteriology*. The same is true of bacteria in the soil, or *soil bacteriology*. He should acquaint himself with relationships of microörganisms to disease, particularly the common diseases of animals; in other words, with *medical* or *pathogenic bacteriology*. He should know something of the ways in which the body resists disease. He must, therefore, study some of the phases of *immunology*. Finally the methods by which disease-producing microörganisms are spread, and the methods by which such passage from one individual to another may be prevented may be considered under the heading of *sanitary bacteriology*.

Bacteriology as a science is of comparatively recent development. It is probable that more has been accomplished, more facts have been discovered relating to microörganisms, since 1900 than in all previous time together. What is true of bacteriology in general is even more true of agricultural bacteriology. Practically all of our information relative to this subject has been gained since 1880.

In the development of the science of bacteriology, five factors have been of major importance, namely, the *development of the microscope;* the *controversy over the theory of spontaneous generation;* the *controversy over the germ theory of fermentation;* the *controversy over pleomorphism among microörganisms;* and finally the *controversy over the germ theory of disease.*

Development of the Microscope.—Microörganisms, and bacteria in particular, are exceedingly minute. Individual cells cannot be seen with the naked eye, and very few observations can be made satisfactorily without the aid of a compound microscope. It is apparent, therefore, that little could be learned concerning bacteria until the microscope had been highly perfected.

Apparently the first authentic record and description of bacteria is that by a Dutch lens maker named van Leeuwenhoek. This investigator succeeded in so combining lenses as to secure magnifications greater than those of his predecessors. In a letter written in 1683 to the Royal Society of London he commented at some length upon his microscopic observations of tartar from teeth and of various decaying materials. Accompanying his paper were drawings of the minute organisms which he observed. These were published in the proceedings of the society, and constitute the first pictures of microörganisms. His observations and pictures are sufficiently accurate so that one familiar with the microörganisms of the mouth can recognize and identify at least some of the forms illustrated with a high degree of probability.

During the next hundred years lenses were improved and compound microscopes came into common use. In 1783 a Danish investigator, Müller, published a study of microorganisms which were observed in various decaying materials and in water. He worked out the first scientific classification of these lower forms of life. Undoubtedly some of the organisms observed were bacteria, and it is to Müller that we owe certain of the common names used in bacteriology, such as vibrio and bacillus.

During the next half century the microscope was still further perfected. Ehrenberg in 1836 greatly elaborated and improved the classification and observations of Müller. A few years later the principle of the oil immersion lens

was evolved, and finally in 1870 the substage condenser developed by Abbé. Microscopes comparable in efficiency to those in use now were unknown before this time. Cohn in 1872 clearly presented the differences between the true bacteria and the protozoa. He placed the bacteria with the plants and worked out a fairly comprehensive and satisfactory classification of genera and species. Since that date advance has been comparatively rapid.

Controversy over the Theory of Spontaneous Generation.—Some of the earlier observers of bacteria and other microörganisms expressed much curiosity as to their origin. It was observed, for example, that if a decoction of vegetables or hay was exposed to the air for a time, the liquid soon swarmed with bacteria. Apparently living things existed where there had been no observed living things before. Many investigators arrived at the conclusion that, unlike higher forms of life, bacteria might originate *de novo*, that is, without preëxisting organisms of the same kind. In fact, they concluded that when there existed a right mixture of water and dead organic material, the right exposure to air, and the right temperature, there was inherent ability on the part of the organic material to change in part to living cells. This point of view was opposed by other scientists, particularly by Pasteur and Tyndall, who contended that microörganisms must always come from preëxisting microörganisms of the same kind, and that there is no proof of spontaneous generation as it concerns bacteria was quite definitely demonstrated. As a result of the laboratory studies on this problem the foundation was laid for modern bacteriology, and many of the methods used in the laboratory to-day were first developed by those who took a leading part in the settlement of this dispute.

The Controversy over the Germ Theory of Fermentation.—Chemistry as a science is somewhat older than bacteriology. Among the subjects which have interested

chemists from early days are the phenomena of fermentation, putrefaction, and decay. It has long been known, for example, that if fruit juices are kept under the right conditions fermentation will occur and alcohol (spirits of wine) is formed. This was early regarded by the chemists as purely a chemical transformation. During the first half of the nineteenth century, however, it was repeatedly observed that alcoholic fermentation was always accompanied by the development of minute or microscopic cells which were called *yeasts*. It was conjectured by those who observed these living cells that they might be the cause of the fermentation. Chemists opposed this conjecture and argued that these minute objects were the results and not the cause of the fermentation. Here again it was necessary to take the subject into the laboratory in order to decide whether microörganisms caused fermentation or were the result of fermentation. The great German chemist Liebig argued for the latter, and Pasteur for the former conception. The controversy between these two schools of thought waged for many years, but was at last settled conclusively by the laboratory experiments of Pasteur. This investigator demonstrated beyond reasonable doubt that practically all fermentation, putrefaction, and decay, are due to the presence and growth of microörganisms, for the most part bacteria, yeasts, and molds. In the course of the controversy, however, many methods of laboratory work were developed, and many new species of microörganisms discovered.

Controversy over the Theory of Pleomorphism.—It is comparatively uncommon in nature to find single kinds or species of bacteria growing by themselves apart from all other species, or as the modern bacteriologist terms it "in pure cultures." This fact was not recognized by some of the earlier investigators, and led to a long argument or controversy over the theory of pleomorphism. About 1870

a physician by the name of Billroth concluded that all bacteria were different growth forms of one single pleomorphic species, which, when growing under different conditions shows different cell forms. His suggestion was made before modern laboratory technic was well developed. Much of the literature, particularly the medical literature, in the two decades following the work of Billroth is greatly confused because of adherence to this doctrine Here again laboratory studies carried out in the most careful manner were necessary to determine the right. They led to the discovery of many new facts regarding microörganisms, and showed quite conclusively that there are many distinct species of bacteria, and that Billroth was mistaken in his assumption that all were growth forms of a single pleomorphic species. It is true that microörganisms sometimes vary in their shape, their appearance, and their size, when grown under different conditions, and sometimes they can be distinguished from each other only with difficulty. Nevertheless there are many distinct kinds, and the theory of pleomorphism in its original form has been disproved.

The Controversy over the Germ Theory of Disease.—The fact that bacteria can produce disease was first adequately demonstrated and proved by Robert Koch in 1872. Before this time there had been occasional conjectures that microörganisms might have something to do with disease but no real proof. Koch examined microscopically the blood of cattle having the disease called anthrax, and observed numerous rod-shaped organisms among the red blood cells. These he succeeded in transferring to culture media and in growing them free from every other kind of organism, that is, in pure culture. He found that he could cultivate them in this fashion indefinitely in the laboratory, and that whenever they were introduced under the skin of sheep or cattle the animal would invariably contract the disease

anthrax and usually succumb. Furthermore, he discovered that in the bodies of animals artificially inoculated there were present the same organisms which he introduced, and that he could get pure cultures again from the tissues of the animal. His proof of the causal relationship of organisms to disease was quite adequate and complete.

Many investigators began studying other diseases and soon bacteria were discovered which caused many of them. These facts were of course in contradiction to the supposed facts which had governed the treatment of disease formerly, and there was much opposition to the acceptance of the statement that bacteria can cause disease. The proof finally became overwhelming, and the whole development of medicine was modified in consequence. The importance of bacteria as causes of disease led to a large amount of study, and gave the needed incentive to the rapid development of the science of bacteriology, and to the modern conception that diseases are of two classes; the so-called *infectious* diseases, which are caused by microörganisms; and the *non-infectious* diseases, which are not so caused. The development of the germ theory of disease led also to a study of the means by which the animal body resists disease, that is, to the development of *immunology*. It resulted also in a study of the means by which disease-producing microörganisms travel from one person to another, that is, to the development of *sanitary bacteriology*.

During the growth and development of the science of bacteriology, and in the laboratory studies necessitated by the various controversies noted, many facts of interest and importance to agriculture were discovered. The knowledge that microörganisms cause fermentation and disease led to a study of foods, particularly of milk and milk products, and to the development of *dairy bacteriology*. A study of bacteria in their relation to the nitrogen of the soil, and the discovery of bacteria living in the roots of leguminous

plants such as clover and alfalfa, laid the foundation for the growth of *soil bacteriology*.

It is apparent from the preceding discussion that the agricultural and technical bacteriologist finds his material in many different fields. Agricultural bacteriology, in other words, cannot be considered as a distinct science, but rather as a compendium of material taken from the various distinct branches of bacteriology, and having agricultural application.

CHAPTER II

GROUPINGS AND GENERAL RELATIONSHIPS OF MICROÖRGANISMS

It has already been noted that four distinct groups of microörganisms are to be considered in agricultural bacteriology, namely, the bacteria, the yeasts, the molds, and a few of the protozoa. It is important that the differences separating these groups from each other should be recognized, and that the position of all in their relationship to other plants and animals should be understood.

Three of these groups, the bacteria, the yeasts, and the molds, are placed in the plant kingdom, the protozoa in the animal kingdom. It is necessary, therefore, first to differentiate microscopic animals from plants.

Differences between Microscopic Plants and Animals.—Bacteria are probably the simplest living plants and protozoa the simplest living animals. Both belong near the bottom in the evolutionary scheme. It is natural, therefore, that many resemblances are to be found between the lower plants and the lower animals. Some investigators, in fact, group all of the unicellular plants and animals together under the name *Protista.* The differentiation between bacteria and protozoa is rendered particularly difficult because there are a few forms which apparently are intermediate, and which are grouped by some investigators with the bacteria and by others with the protozoa. Nevertheless, most forms in the two groups are not difficult of differentiation. Plant cells usually possess a firm and well-defined cell wall, while the protozoa, at least during their active development, usually do not. The bacteria, in gen-

eral, have the attributes of plant cells. They multiply by transverse fission, while many of the protozoa multiply by fission which is either longitudinal or at least not at right angles to the longest axis of the cells. Bacteria are never amoeboid in their movements as are protozoa of many types. Furthermore, many intergrading forms exist between the true bacteria and that group of plants known as the blue-green algae. No careful student of bacterial morphology can escape the conclusion that the relationships are probably closest to this group of plants, although a few of the bacteria show close resemblances and relationships to certain of the mold fungi.

Both from the standpoint of structure and of connecting forms, the bacteria on the whole show closer relationship to the plants than to the animals or protozoa.

Confusion sometimes arises because of the fact that many of the bacteria are actively motile. It should be recognized that the ability to swim about is not a characteristic peculiar either to plants or to animals. Many forms which are definitely known to be plants possess this power, the sperm cells, for example, of even some of the higher forms of plants such as mosses, ferns and cycads, can move independently. A misunderstanding of this fact led to some confusion in early studies of bacteria, for it is only within the last half century that their true relationships have been understood, and they have been placed definitely with the plants.

Position of Bacteria, Yeasts, and Molds in the Plant Kingdom.—Botanists generally recognize four great subdivisions of plants, the seed plants or *Spermatophyta,* the fern plants or *Pteridophyta,* the moss plants or *Bryophyta,* and the thallus plants or *Thallophyta.* The bacteria, yeasts, and molds all belong in this last group.

The *Thallophyta* are separated from other plants in that they do not develop a complex plant body with differenti-

ation into roots, stem and leaves. Sometimes the entire plant consists of a single cell, that is, is unicellular. In other cases it may consist of a chain of cells united to form more complex structures, though this is never the case with bacteria, yeasts, or molds. The group *Thallophyta* has three important subdivisions. The first is that of the *algae*, forms containing green coloring matter or chlorophyll. The second group is that of the *fungi* which differ from the *algae* in that they do not possess chlorophyll. It includes such forms as yeasts, molds, puffballs, mushrooms, mildews, etc. The third subgroup is that of the *Schizophyta* or fission plants in which multiplication is of some other type than that of simple cell fission. One of the subdivisions of this group is that of the *Schizomycetes* or bacteria. In the following scheme of classification an attempt is made to show the various important subgroups of the *Thallophyta*. Those subgroups which contain organisms to be studied in bacteriology are designated by **bold-faced type.**

GROUPS OF THALLOPHYTA SHOWING POSITION AND RELATIONSHIPS OF BACTERIA, YEASTS, AND MOLDS

Thallophyta.—Simple plants, never differentiated into roots, stems and leaves.

A. Unicellular plants, multiplying by cell fission only.
   1. Without chlorophyll. Bacteria or **Schizomycetes.**
   2. With chlorophyll. Blue-green algae or *Schizophyta.*

B. Unicellular or multicellular. Multiplying by means other than simple cell fission.
   1. Without chlorophyll, the fungi, including the **yeasts, molds,** *mildews, smuts, rusts, puffballs, mushrooms,* etc.
   2. With chlorophyll, including the *seaweeds, pond scums, water silks,* etc.

Differentiation of Bacteria, Yeasts, and Molds.—The bacteria and yeasts are readily differentiated from molds in that they are always unicellular while the molds, in general, are multicellular. Bacteria always multiply by fission, that is, the cells divide at right angles to their longest axis. Yeasts, in general, multiply by budding, although a few yeasts are known to multiply by fission. When bacteria produce spores, usually but one spore is found in a single cell, while with yeasts there is usually more than one. This is always true in those yeasts which multiply by fission. There are also some minute structural differences which may usually be observed between yeasts and bacteria. The yeast cell shows a definite nucleus while the presence of a definite nucleus is not so clearly established for bacteria. In most cases the yeast cells are decidedly larger than those of bacteria.

Botanists do not recognize a distinct group which they term molds. Organisms belonging to this group, which is convenient for the purposes of the bacteriologist, are really members of different groups of fungi. They all resemble each other, however, in forming more or less cottony or velvety masses of fungus threads from which spore stalks in various shapes and spores in various groupings are produced. A few of the molds show forms which intergrade with the bacteria. Multiplication in molds is usually by means of spores which are produced in large numbers by single plants.

The Protozoa.—The protozoa are the one-celled animal forms existing in a great variety of shapes, sizes, and methods of life in soil, in water, and occasionally producing diseases in man and animals. The group is a very large one. From the standpoint of agricultural bacteriology we are interested in the group because of the possible importance of some forms in soils and the undoubted significance of others in the production of diseases.

CHAPTER III

MORPHOLOGY AND CLASSIFICATION OF THE BACTERIA

Morphology of Bacteria

Shape.—Bacterial cells are usually one of four shapes, straight rods, curved rods, spheres, or filaments. A straight rod is termed a *bacillus;* a curved rod a *spirillum;* a spherical cell a *coccus;* the filamentous forms are sometimes termed *trichobacteria.*

Bacteria not infrequently develop cells which are not of the normal size and shape. In some cases these are prob-

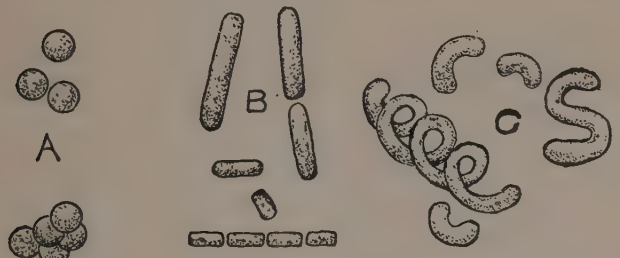

Fig. 1.—Shapes of bacteria. A. Spherical bacteria (cocci). B. Rod-shaped bacteria (bacilli). C. Spiral bacteria (spirilla).

ably degenerate types, cells produced as a result of growing under abnormal or unfavorable conditions. In other cases they may represent special developmental phases of the life history of the organism. In general, such unusual cells are termed *involution forms.* Some authors insist that this term should be used only for those which represent degenerate types and not those which represent phases in the normal life cycle.

Size.—Bacteria are the smallest plants. The microscope, and usually the compound microscope, is necessary for the study of the individual cells. It is, therefore, convenient to use the microscopic unit of measurement. The micron (abbreviated μ) is defined as one one-thousandth of a millimeter, or one ten-thousandth of a centimeter, or approximately one twenty-five-thousandth of an inch. Most bacteria are 0.5 to 5μ in diameter, and from 0.5 to 10μ in length. Probably the average bacterial cell does not have a volume much greater than one cubic micron. The minuteness of these organisms is emphasized when it is recalled that there are one trillion cubic microns in a cubic

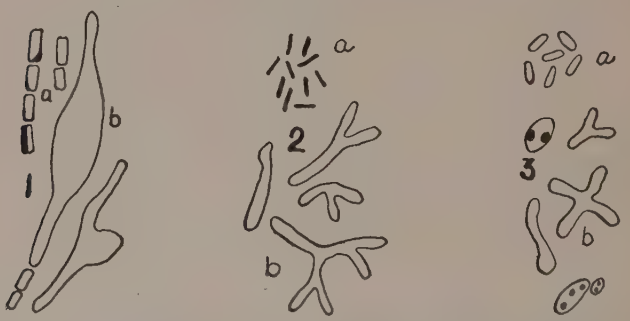

FIG. 2.—INVOLUTION FORMS. a. Normal shapes. b. Involution forms. 1. *Acetobacter*. 2. *Mycobacterium*. 3. *Rhizobium*.

centimeter, therefore, approximately this number of bacteria of average size would be required to fill a space of one cubic centimeter. The specific gravity of the cell is usually only slightly greater than that of water. It would require, therefore, nearly a trillion of such bacteria to weigh a gram. When water is heavily polluted it may sometimes contain one million bacteria per cubic centimeter. This number of bacteria, of average size, would not occupy more than one millionth of the space within that cubic centimeter.

Multiplication of Bacteria.—Bacteria multiply by a process of simple fission. A rod elongates to about double its original length and splits in the middle to form two individuals. Spherical cells frequently elongate somewhat before they divide.

Bacteria in many cases may multiply with considerable rapidity. Certain of the common bacteria, for example, when placed under the most favorable conditions for growth, that is, with an abundance of food, moisture, the right temperature, and all conditions optimum, may grow to their full size and divide once every twenty minutes. Probably most of the more active bacteria can grow to their full size and divide to form two individuals within one-half hour. If it is assumed that this process continues for two days the number of bacteria will be 2^{96}, a number having some twenty-eight figures. A rough calculation of the weight of such a bacterial mass will show it to be more than a trillion tons. Obviously no such bacterial masses can ever be formed, inasmuch as the optimum conditions for growth cannot long continue. The principal factors which limit the rapidity of growth are the disappearance of the available food material, and the production of substances by the bacteria which are more or less harmful to their own development. This rapidity of multiplication, however, explains why fermentative changes, such as the souring of milk, may take place so rapidly. A comparatively small number of bacteria introduced into a bottle of milk and kept at a suitable temperature will cause it to become sour within a few hours.

Cell Groupings of Bacteria.—Bacterial cells do not always become detached from each other immediately following cell division. In many cases the presence of capsules or of mucilaginous or gummy walls may cause the cells to cling together. It is obvious that rod-shaped bacteria, inasmuch as they can divide or multiply only by splitting

crosswise, must occur either as isolated cells or in chains. Such an organism is sometimes termed a *streptobacillus*. Spiral bacteria very rarely occur in groups though occasionally they are found in chains.

It has been stated that bacteria always divide at right angles to the longest axis of the cell. Spherical cells, or cocci, have no longest axis. There is, therefore, *a priori* no reason why they cannot divide in any plane. Some spherical cells show no regularity in the manner in which they divide. If such cells cling together they will form an irregular or grape-clusterlike mass. Such an organism is termed a *staphylococcus*. Some cocci show a decided tendency to remain united in pairs. An organism of this type is termed a *diplococcus*. When spherical cells divide persistently in parallel planes a chain of cocci will be produced. Such

FIG. 3.—FORMS AND GROUPINGS OF THF COCCI. 1. Single or isolated cells. 2. Irregular masses. 3. Pairs. 4. Chains. 5. Plates. 6. Packets or cubes.

an organism is termed a *streptococcus*. A few kinds of cocci have been described which divide alternately in two planes forming first pairs, and then squares of cells. An organism of this type may be termed a *pediococcus*. Still others divide in three planes, each plane at right angles to the other two. This will result in the formation first of pairs, then of squares, then of cubes of bacteria. Such a cubical collection of bacteria is termed a *sarcina*.

It should be noted that the names given above are not the scientific names of genera, but are common descriptive terms. It will be found later that some of these same words

are used also as generic names. When used in a simple descriptive sense they are not capitalized. When used as the names of genera they are capitalized and are generally written in italics.

Structure of the Bacterial Cell and Its Appendages.—The bacterial cell is a comparatively simple structure. It possesses a definite *cell wall.* This may be surrounded by various layers of gelatinous material or by membranes, termed *capsules* or *sheaths.* It contains the *protoplasm* or living cell material and various other cell inclusions, and it may be motile by means of *flagella.*

The Cell Wall.—Each bacterial cell is surrounded by a relatively firm membrane or cell wall. Its composition apparently is not uniform among all bacteria. In a few cases the cell wall will give the microchemical reaction charac-

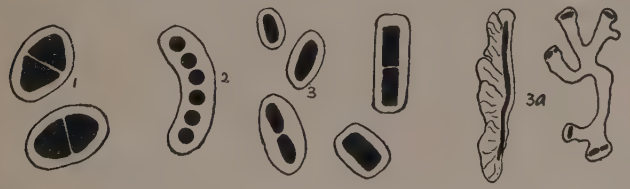

FIG. 4.—CAPSULES OF VARIOUS BACTERIA. 1. Capsulated diplococcus. 2. Capsulated streptococcus. 3. Capsulated bacilli. 3a, Bacilli with asymmetric capsules.

teristic of cellulose, indicating a close relationship in composition to the cell walls of higher plants. Most bacteria are supposed to have cell walls which are somewhat *chitinous,* that is, they are nitrogenous in nature. Chitin is closely related to the carbohydrates. When hydrolyzed it breaks down into glucosamine and acetic acid. The cell wall generally is thin and layers cannot be differentiated.

Cell Capsules and Sheaths.—Some species of bacteria secrete large amounts of gelatinous material about the cell. In some cases this dissolves relatively rapidly in the medium in which the organism is growing, sometimes making it

slimy or gelatinous in consistency. In other cases this material remains attached to the cell, forming a layer of greater or less thickness. This may be regarded as the outer portion of a greatly swollen cell wall. Such a mass is termed a *capsule*. This capsule is usually composed of gum or gumlike material. Occasionally it is nitrogenous in nature.

A few filamentous or rod-shaped bacteria growing in water surround themselves by a relatively firm membrane, a tube in which the filaments or chain of cells lie. This tube is termed a *sheath*. In some species this sheath becomes impregnated with iron.

Protoplasm and Cell Inclusions.—The protoplasm or living material within the bacterial cell is usually not clearly differentiated as in the higher plants into nucleus and cytoplasm. It is probable that in most cases nuclear material, that is, chromatin, is scattered more or less throughout the protoplasm. Some bacteria apparently have a primitive type of nucleus.

The outermost portion of the protoplasm, that is, that portion lying next to the cell wall, is differentiated as a membrane termed the *ectoplast*. This ectoplast apparently functions in determining what can enter and what can leave the cell.

Bacterial cells may contain inclusions of many kinds. Most common are granules which stain relatively deeply with some of the ordinary laboratory dyes such as methylene blue. Because their color reactions resemble chromatin they are termed metachromatic granules. In some species of bacteria *vacuoles* are developed. These apparently are spaces in the protoplasm filled with cell sap but not with living material. Each vacuole is surrounded by a membrane of the same general type as that surrounding the entire mass of protoplasm. Granules of carbohydrate are observable in some bacteria. The exact composition is not

well understood, but in some cases it is apparent that the reserve carbohydrate food material resembles glycogen, in others it is perhaps more closely related to starch. Granules which give the starch color reaction with iodine have been termed *granulose*. Certain bacteria which live in water containing hydrogen sulphide show *sulphur* granules in the protoplasm. It is possible that in some cases *oil drops* may be detected.

Flagella.—A very few species of cocci, many species of bacilli, practically all species of spirilla, and none of the filamentous bacteria, possess organs of locomotion termed *flagella*. These are exceedingly slender, so slender in fact that they are observed under the microscope usually only by the most careful staining methods. They may be re-

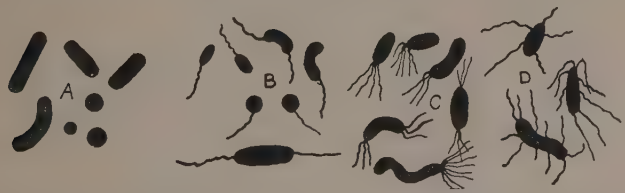

FIG. 5.—FLAGELLA OF BACTERIA. A. Non-flagellate or atrichous bacteria. B. Bacteria with single polar flagellum, monotrichous. C. Bacteria with clusters of polar flagella, lopotrichous. D. Bacteria with flagella on all sides, peritrichous.

garded as differentiated protoplasmic organs projecting through the cell wall to the outside. They are relatively long, sometimes many times the length of the cell which produces them. By means of a spiral or corkscrew motion these flagella propel the organism.

Those species of bacteria which do not possess flagella are said to be *atrichous*. Some species possess a single flagellum at one end of the cell. Such organisms are termed *monotrichous*. Organisms having a tuft of flagella at one end are termed *lophotrichous*. Those bacteria which have flagella distributed over the entire surface of the cell are said

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to be *peritrichous.* Some investigators recognize another group termed *amphitrichous,* that is, cells having flagella at both ends. Such organisms, however, are in reality the same as those having flagella at one end only. When a monotrichous or lophotrichous motile cell is about to divide, flagella frequently develop at the other end so that each daughter cell, following the division of the mother cell, will be motile. The flagella ordinarily point in the direction in which the organism is swimming, that is, they pull the organism about rather than push it.

Spore Formation.—A spore may be defined as a cell or group of cells set apart specifically for purposes of reproduction. Usually, though not invariably, spores are more resistant to unfavorable conditions than the cells which produce them.

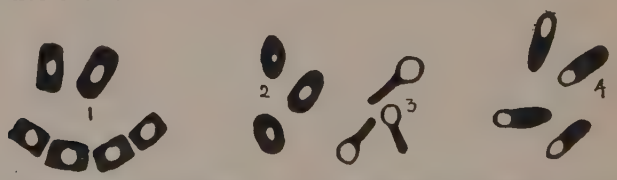

FIG. 6.—SPORES OF BACTERIA. 1. Spores centrally located, cells not swollen. 2. Spores central, cells swollen. 3. Spores polar, cells swollen. 4. Spores polar, cells not swolllen.

Certain kinds of bacteria may produce spores on the inside of the cells. With the exception of two or three little known species a bacterial cell produces only a single spore. Because of their location bacterial spores are sometimes termed *endospores.* It is apparent that inasmuch as a single spore forms within a cell, spore production among bacteria does not involve multiplication or increase in numbers.

None of the common species of cocci or spirilla produce spores, but some species of the rod-shaped organisms or bacilli do.

The first evidence of spore formation in the bacterial cell is usually the appearance of certain granules. Gradually

the protoplasm at the point where the spore is to form becomes more dense and finally surrounds itself by a definite membrane or membranes. A mother cell containing a spore is sometimes termed a *sporangium*. The spore differs from the cell in which it is produced usually by being somewhat smaller, by having a much firmer membrane, and by its lower water content. It is usually well fitted to survive unfavorable conditions for a considerable period of time. In most cases the spore mother cell wall eventually breaks away leaving the spore free.

Usually each species of spore-producing bacillus has its own characteristic shape, size, and position for the spore. These spores may be located either at the center or at or near the end of a cell. In some species in which the spore is located near the equator the mother cell wall bulges, making the cell spindle-shaped. Such an organism is sometimes termed a *clostridium*. In other cases the spore does not cause such cell enlargement. A few species of bacteria produce spores at the end of the cell, causing considerable enlargement. Such a drumstick-shaped cell is sometimes termed a *plectridium*.

When spores come under favorable conditions for development they will germinate, producing rods similar to those from which they originated. These rods will then go on multiplying by fission until stimulated in some way themselves to produce spores. The stimulation to spore production is not necessarily unfavorable conditions for growth as is ordinarily understood. In other words, although spores undoubtedly serve the purpose of tiding bacteria over unfavorable conditions, the organism does not need to be subjected to these unfavorable conditions before producing spores. It appears to be quite as much a part of the regular life cycle of some bacteria to produce spores as it is a part of the regular life cycle of the corn plant to produce an ear of corn.

Conidia Formation.—Certain of the filamentous or so-called higher bacteria sometimes divide by numerous cross walls and split into small cells which function as spores. These are termed *conidia.* They are probably not commonly found among the cocci, bacilli, or spirilla.

Classification of Bacteria

The true bacteria belong to the class *Schizomycetes.* They may be characterized as minute, one-celled, chlorophyll free, colorless, rarely violet-red or pigmented plants, which typically multiply by dividing in one, two, or three directions of space. The cells thus formed are usually spherical, cylindrical, comma-shaped, spiral or filamentous, and are often united into filamentous, flat, or cubical aggregates. Filamentous species are often surrounded by a common sheath. The cell plasma is generally homogenous without a definite nucleus. Sexual reproduction is absent. In some species reproductive bodies are produced, either endospores or conidia. The cells in some species are motile by means of flagella.

The class *Schizomycetes* may be subdivided into six distinct orders. Of these, four are not of great agricultural significance. Two, however, are of sufficient interest to require discussion. The four orders not further discussed are the slime bacteria (belonging to the order *Myxobacteriales*), the sulphur bacteria (belonging to the order *Thiobacteriales,*) the iron bacteria (belonging to the order *Chlamydobacteriales*) and the spirochetes (*Spirochaetales*). The two orders of agricultural significance are the filamentous or branching bacteria (belonging to the order *Actinomycetales*) and the true bacteria (order *Eubacteriales*).

Orders in botany are divided into families, families into genera, and genera into species. Each kind of bacterium constitutes a species. The related species are grouped together into genera, and related genera into families. For

example, there is one type of rod-shaped organism which produces typhoid fever, another type somewhat closely related to it which causes dysentery. These two species are brought together in the genus *Bacterium*. This genus, with several other genera of bacteria, are united into the family *Bacteriaceae*, and this family again united with others to form the order *Eubacteriales*.

The rules which govern the giving of scientific names to plants and animals should be recognized also in bacteriology. To every kind of organism a name should be given consisting of two words: the first of these a proper name, always capitalized, the name of the genus; the second name is the name of the species. This latter name is never capitalized unless it is derived from a proper noun. All genus and species names are in Latin. The name of the organism which causes typhoid fever is *Bacterium typhosum*. Frequently the name of the man who first described the organism under the particular name accepted is given also, thus, *Bacterium coli* Escherich, indicates that Escherich first applied this name to the organism called the colon bacillus. Sometimes investigators have named species but have placed them in the wrong genus. The man who first recognized the species may have his name written in parentheses between the name of the species and the name of the individual who first used the right combination, for example, an organism sometimes associated with pus production in animals is *Pseudomonas aëruginosa* (Schroeter) Migula. This indicates that *Schroeter* first used the name *aëruginosa* but that Migula placed this species in the genus *Pseudomonas*.

The species name of an organism should ordinarily consist of a single word. Some bacteriologists have not followed this rule and have given complex Latin names to organisms on the theory that a name should be a description, for example, one species was termed *Bacillus saccharo-*

butyricus fluorescens liquefaciens immobilis. The author was indicating that he was dealing with a rod-shaped organism which fermented sucrose by the formation of butyric acid, produced a fluorescent pigment in artificial media, liquefied gelatin, and was nonmotile. It is quite as unnecessary to use complex names of this type as it is to have the Christian name applied to an individual necessarily descriptive of that individual.

Many microörganisms have received common names. These should not be confused with the true scientific names even though they somewhat resemble them, for example, the organism which causes epidemic pneumonia is termed the pneumococcus. Its true scientific name is *Diplococcus pneumoniae.*

Many of the genera of bacteria belonging to the two orders to be studied are not of sufficient economic importance to warrant discussion in this connection. Only genera which contain species that bring about noteworthy changes are included in the discussion.

First there is presented an artificial key for the differentiation of the economic genera. This is followed by the classification of genera and families recommended by the Committee on Nomenclature of the Society of American Bacteriologists.

It will be noted in the key and in the description of genera and other groups that it is sometimes necessary to use physiological rather than morphological criteria for differentiation. For example, Gram's staining method (discussed in a later chapter) results in coloring some organisms and leaving other organisms unstained. Some organisms can produce acid or acid and gas from certain carbohydrates, other forms cannot. This fact is of great value in separating related groups. Occasionally the type of growth upon culture media may be of importance.

Key to Genera of Bacteria of Agricultural and Industrial Significance

A. Typically never filamentous nor producing a mycelium.
  B. Cells spherical.
    C. Cells occurring typically in chains.
      D. Cells forming gelatinous masses in sugar media, saprophytic. ..... *Leuconostoc.*
      DD. Parasitic. Cells not forming zoögloeal masses. ..... *Streptococcus.*
    CC. Cells not in chains.
      D. Cells typically in pairs.
        E. Cells Gram-negative. Uusually coffee-bean-shaped. ..... *Neisseria.*
        EE. Cells Gram-positive. Usually lanceolate. ..... *Diplococcus.*
      DD. Cells not in pairs.
        E. Typically parasitic. Cells in irregular groups. ..... *Staphylococcus.*
        EE. Typically saprophytic.
          F. Cells arranged in regular packets. *Sarcina.*
          FF. Cells not arranged in packets, not regularly grouped.
            G. Pigment usually yellow. ..... *Micrococcus.*
            GG. Pigment red. ..... *Rhodococcus.*
  BB. Cells elongate, not spherical.
    C. Rods not spiral or curved.
      D. Cells acid fast. ..... *Mycobacterium.*
      DD. Cells not acid fast.
        E. Endospores present.
          F. Aërobic. ..... *Bacillus.*
          FF. Anaërobic. ..... *Clostridium.*
        EE. Endospores absent.
          F. Not growing on ordinary organic media, oxidizing ammonia or nitrites.
            G. Oxidizing ammonia. ..... *Nitrosomonas.*
            GG. Oxidizing nitrous acid. ..... *Nitrobacter.*
          FF. Not securing growth energy by oxidation of ammonia or nitrites.
            G. Oxidizing carbon compounds aërobically, saprophytic.
              H. Oxidizing alcohol to acetic acid. *Acetobacter.*
              HH. Oxidizing carbohydrates. Fixing atmospheric nitrogen.
                I. Cells relatively large. Free living soil bacteria. ..... *Azotobacter.*
                II. Cells smaller. Symbiotic in roots of leguminous plants. *Rhizobium.*

GG. Not securing growth energy exclusively by the aërobic oxidation of carbon compounds.
I. Usually producing a fluorescent pigment. Usually motile by polar flagella ..... *Pseudomonas.*
II. Without fluorescent pigment. Motile or nonmotile, if the former, flagella peritrichous.
J. Rods of irregular shape, sometimes clubbed or branched. Nonmotile animal parasites.
K. Gram-positive rods, frequently granular or clubbed. *Corynebacterium.*
KK. Gram-negative, rods sometimes elongated or branched. Not granular. ..... *Pfeifferella.*
JJ. Rods relatively regular, and staining evenly or bipolar. Motile or nonmotile.
K. Usually showing bipolar staining. Weak power of fermentation. ..... *Pasteurella.*
KK. Bipolar staining absent.
L. Growing only or best in presence of hemoglobin. *Hemophilus.*
LL. Not requiring hemoglobin.
M. Saprophytic red or violet forms.
N. Pigment red. ..... *Erythrobacillus.*
NN. Pigment violet. .. *Chromobacterium.*
MM. Without red or violet pigment.
N. Gram-positive lactic acid bacteria. ..... *Lactobacillus.*
NN. Gram-negative.
O. Producing plant diseases. ..... *Erwinia.*
OO. Not producing plant diseases.
P. Rods not uniform. Ferment sucrose not lactose. ..... *Proteus.*
PP. Rods uniform. Never ferment sucrose except that lactose is also fermented. *Bacterium.*

CC. Rods curved.
D. Short, comma-shaped. ..... *Vibrio.*
DD. Longer, spiral. ..... *Spirillum.*

Outline of Bacterial Classification

It was noted above that two only of the five orders of bacteria will be discussed: the order of the true bacteria or *Eubacteriales,* and the order of the thread bacteria or *Actinomycetales.*

Order Eubacteriales

This order contains the forms usually termed the true bacteria, that is, those which are considered least differentiated and least specialized. They do not require hydrogen sulphide or other sulphur compounds in abundance, and the cells do not contain either sulphur granules or bacteriopurpurin. The cells may be motile by means of flagella or nonmotile, but they are never notably flexuous. Cell multiplication is always by transverse fission. Some of the genera, particularly among the rod-shaped types, produce endospores. The cells are usually minute and spherical, rod-shaped, or spiral in shape, not commonly producing true filaments, and never branching except in the so-called involution forms. This order includes most of the common bacteria. Altogether seven families belonging to order *Eubacteriales* are recognized. The following key may be used for the differentiation of the families:

Key to Families of the Eubacteriales

A. Securing growth energy by direct oxidation of carbon, hydrogen or nitrogen or their compounds. Aërobic. Cells rod-shaped or occasionally spherical.	I. *Nitrobacteriaceae.*
AA. Not securing growth energy solely as in preceding.	
B. Cells producing endospores.	II. *Bacillaceae.*
BB. Cells not producing endospores.	
C. Cells spherical.	
CC. Cells not spherical.	III. *Coccaceae.*
D. Cells rod-shaped.	

E. When motile, with polar flagella, frequently producing a fluorescent or a yellow pigment. IV. *Pseudomonadaceae.*
EE. Not as E. Flagella when present, peritrichous. ..... V. *Bacteriaceae.*
DD. Cells bent or spiral. ..... VI. *Spirillaceae.*

I. Family Nitrobacteriaceae

Bacteria belonging to this family are usually rod-shaped, occasionally spherical. In some genera the cells are motile and in others nonmotile. In a few genera there is a decided tendency toward the formation of threadlike or branched involution forms. Endospores are never produced. These bacteria are grouped together primarily because they must grow in the presence of free oxygen, and secure their growth energy by the direct oxidation of carbon, or hydrogen, or simple compounds of these elements and of nitrogen. None produce disease, and with the exception of the genus which lives in the nodules on the roots of leguminous plants, none are parasitic.

The genera of sufficient agricultural interest to be discussed are *Acetobacter, Nitrosomonas, Nitrobacter, Azotobacter* and *Rhizobium.* In addition to these genera three others [1] have been described.

Acetobacter.—In this genus the organisms are rod-shaped, frequently in chains, and usually nonmotile. The bacteria grow on the surface of alcoholic solutions, securing the energy for their growth usually by the oxidation of alcohol to acetic acid, or occasionally by the oxidation of sugars to simpler compounds. Involution forms are not infrequent, occurring as filamentous, club-shaped, and even branched cells. Organisms belonging to this genus constitute the so-called *mother of vinegar* which forms over

[1] The genus *Hydrogenomonas* secures its energy by the oxidation of free hydrogen, *Methanomonas* by the oxidation of methane, *Carboxydomonas* by the oxidation of carbon monoxide.

the surface of cider or wine in the process of vinegar manufacture.

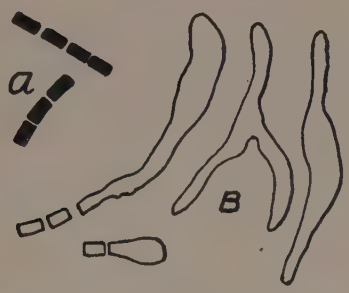

FIG. 7.—ACETOBACTER. A. Normal cells. B. Involution forms.

Nitrosomonas.[1]—Bacteria belonging to this genus have cells either rod-shaped or spherical, motile or nonmotile. These organisms are soil forms securing their growth energy by the oxidation of ammonia to nitrous acid. They do not require organic compounds for growth. They are

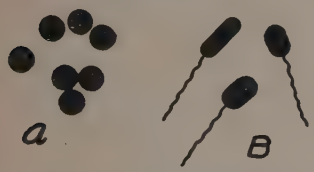

FIG. 8.—NITROSOMONAS. .A. Non-motile spherical type. B. Motile, rod-shaped type.

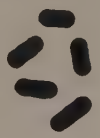

FIG. 9.—NITROBACTER.

responsible in the soil for initiating the changes later to be discussed under the heading of "nitrification."

Nitrobacter.—The organisms belonging to this genus are rod-shaped, nonmotile, and do not require the presence of organic matter for growth. They are commonly present in the soil and secure the energy for growth by the oxidation of nitrites to nitrates. They complete the process of

[1] This genus includes the organisms sometimes described as *Nitrosococcus*.

"nitrification." This genus, together with the preceding genus, are of considerable importance in determination of soil fertility.

Azotobacter.[1]—Organisms belonging to this genus are relatively large rods, sometimes spherical, and occasionally almost yeastlike in appearance. They are typically soil forms securing their growth energy by the oxidation of carbonaceous material, and of unusual importance because of their ability to fix atmospheric nitrogen for their own

FIG. 10.—AZOTOBACTER.

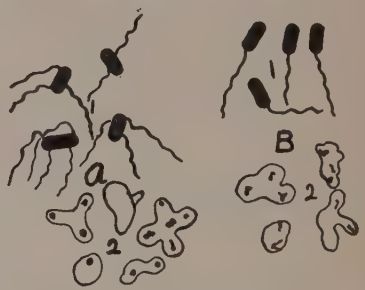

FIG. 11.—RHIZOBIUM. A. Species motile by peritrichic flagella. 1. Young motile cells. 2. Involution forms. B. Species motile by means of polar flagella. 1. Young cells. 2. Involution forms.

use, thus increasing the nitrogen content of soils. They do not live upon the roots of leguminous plants, but free in the soil. In the laboratory their ability to use atmospheric nitrogen may be shown by growing them in solutions containing carbohydrates but deficient in nitrogen compounds.

Rhizobium.—These organisms are minute rods, motile when young, by means of flagella which may be either polar or peritrichous. The cells may occur free in the soil, but usually are present in the characteristic nodules on the

[1] Other names sometimes used for *Azotobacter* are *Parachromatium*, and *Azotomonas*.

roots of leguminous plants. By use of the energy secured from the oxidation of carbon compounds, usually sugars, they are capable of fixing atmospheric nitrogen. The species found generally upon the roots of leguminous plants is *Rhizobium leguminosarum.*[1]

It is capable of fixing atmospheric nitrogen when grown in the laboratory in solutions containing carbohydrates.

II. Family Bacillaceae

The organisms belonging to this group are always rod-shaped, all produce endospores, and the flagella when present are peritrichous. For the most part they are able to decompose complex organic compounds, and are active in producing decay in nature. Two genera are included in this family; the genus *Bacillus,* including those forms which require free oxygen for their development; and *Clostridium,* including those forms which do not grow in the presence of free oxygen.

Bacillus.—The organisms belonging to this genus are all

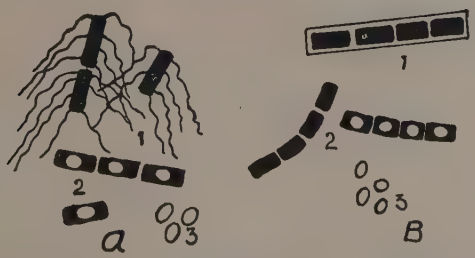

Fig. 12.—Bacillus. A. Motile species. 1. Vegetative cells. 2. Sporulating cells. 3. Free spores. B. Nonmotile species. 1. Vegetative cells in capsules. 2. Left. Noncapsulated vegetative cells. Right. Sporulating cells. 3. Free spores.

rod-shaped, sometimes occurring in chains. Under the right conditions these bacteria are among the most active

[1] Many other names have been applied to this organism. It is frequently discussed in literature as the *Bacillus radicicola.*

in nature, producing decomposition of organic materials. One species produces the disease anthrax in animals and man, and one or two species produce the diseases termed *foul brood* of bee.

Clostridium.—The organisms of this genus are all rod-shaped, producing endospores, but not growing in the presence of free atmospheric oxygen. Frequently the rods are swollen at time of spore production, producing spindle-shaped or club-shaped cells. Some of the bacteria of this group are among the most active of the putrefactive forms,

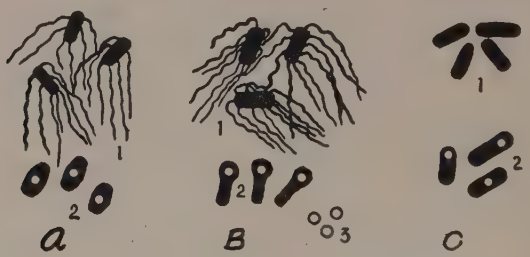

FIG. 13.—CLOSTRIDIUM. A. Motile species. 1. Vegetative cells. Sporulating, swollen cells, spores equatorial. B. Motile species. 1. Vegetative cells. 2. Sporulating cells, spores, polar, cell swollen. 3. Free spores. C. Nonmotile species. 1. Vegetative cells. 2. Sporulating cells, cells not swollen.

particularly in the production of malodorous compounds such as butyric acid. Other species are capable of producing disease, especially when introduced into wounds. Among the diseases produced are *gaseous gangrene* in man, *malignant edema*, *blackleg*, and *bradsot* in animals, and *tetanus* in man and animals.

III. FAMILY COCCACEAE

The organisms belonging to this family have cells usually spherical when free, though during division they may be

somewhat elliptical. If the cells remain in contact after division they are frequently flattened at the surface of contact. They may form chains, packets or irregular masses. Very few cocci are motile; none of the motile species are of economic importance. Spores are not produced. Most of the genera of this family are parasitic, many species are disease-producing. A few are of importance because of the fermentative changes which they bring about. The most important genera are *Neisseria, Streptococcus, Diplococcus, Staphylococcus, Micrococcus, Sarcina.*[1]

Neisseria.—The organisms of the genus are strict parasites, usually growing rather poorly in artificial culture media. The cells usually occur in pairs, flattened at the proximal sides, usually coffee-bean-shaped, and Gram-negative. Two of the diseases produced by organisms of this

FIG. 14.—NEISSERIA.

FIG. 15.—STREPTOCOCCUS.

group are of importance, namely, cerebrospinal meningitis, and gonorrhea.

Streptococcus.—This genus includes those spherical bacteria whose cells occur normally in chains. For the most part the cells are Gram-positive. Certain of the species are important in that they bring about lactic acid fermenta-

[1] Other genera are: *Leuconostoc*, forming large gelatinous masses in sugar solutions; and *Rhodococcus*, including the cocci which produce a red pigment.

tion in milk. Others are capable of producing diseases. Certain species, for example, are commonly associated with tonsillitis, rheumatism, with the disease strangles in the horse, wound infection, and pus production.

Diplococcus.—The organisms belonging to this genus are strict parasites, Gram-positive, occurring in pairs, the cells usually somewhat pointed. The most important species is the organism usually associated with pneumonia.

FIG. 16.—DIPLOCOCCUS. Capsulated.

FIG. 17.—STAPHYLOCOCCUS

Staphylococcus.—In this genus the spherical cells are united in more or less irregular masses. They are Gram-positive, and usually parasitic. They are commonly associated with pus production, wound infection, the development of boils, abcesses, and similar conditions in the bodies of man and animals.

FIG. 18—MICROCOCCUS.

Micrococcus.—The organisms of this genus are usually not parasitic, but are present commonly in water and soil. They are frequently Gram-negative, occasionally motile, and when grown in the laboratory usually develop a yellow pigment. There are no species of considerable economic importance.

Sarcina.—Organisms belonging to this genus have their spherical cells usually massed in regular packets, consisting of cubes of eight cells each. When grown in the laboratory pigment is frequently produced, usually yellow in color.

IV. Family Pseudomonadaceae

This family contains a single genus, *Pseudomonas*. The cells are rod-shaped, and usually motile by means of polar flagella. Frequently a fluorescent green or brown pigment is produced in culture media. In other species an insolu-

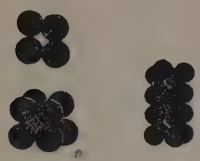

Fig. 19.—Sarcina.

Fig. 20.—Pseudomonas.

ble yellow pigment is formed. Among those producing a green fluorescent pigment is the *Pseudomonas aëruginosa*, an organism sometimes found in wounds. Many of the yellow and a few white species produce diseases in plants.

V. Family Bacteriaceae

Fig. 21.—Erythrobacillus

This is the largest of the families of bacteria. It includes those rod-shaped bacteria whose cells are usually regular in shape, do not produce endospores, and when motile do not have polar flagella only. In most genera the cells are gram-negative. Fluorescent pigment is not produced. The important genera are *Erythrobacillus*, *Erwinia*, *Proteus*, *Bacterium*, *Pasteurella*, *Hemophilus*, and *Lactobacillus*.

Erythrobacillus.—The organisms of this group are small aërobic bacteria, producing a red or pink coloring matter, sometimes yellow or orange. Some species are motile. The most important species is *Erythrobacillus prodigiosus*.

This is found to cause red spots in bread and other carbohydrate foods.[1]

Erwinia.[2]—The organisms belonging to this genus all produce disease in plants. Their growth in the laboratory is usually whitish. They form acids in certain carbohydrates, but as a rule no gas. One of the most important species is *Erwinia amylovora,* the organism causing pear blight.

Proteus.—The organisms belonging to this genus are highly pleomorphic rods, frequently producing filaments and curved cells as involution forms. They are Gram-negative and actively motile. Upon suitable culture media they form amoeboid colonies. The organisms ferment glucose and sucrose but not lactose with the formation of acid

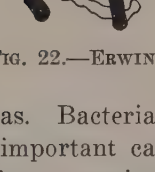

FIG. 22.—ERWINIA.

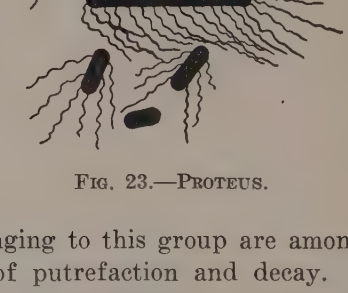

FIG. 23.—PROTEUS.

and gas. Bacteria belonging to this group are among the most important causes of putrefaction and decay. They have been occasionally found associated with disease.

Bacterium.—The organisms belonging to this genus are Gram-negative rods, frequently motile, and easily cultivable. Most species ferment certain carbohydrates with the formation of acid and frequently of gas. They are typically intestinal parasites in man and higher animals. Some

[1] Another genus belonging to this family is *Chromobacterium* in which the cells produce a violet or blue pigment.

[2] This genus is variously incorporated by writers in plant pathology with the genus *Bacterium* or the genus *Bacillus.*

species are not infrequent in the soil. A few species are pathogenic, including the organisms causing *typhoid fever, paratyphoid fever, dysentery,* and certain types of *food poisoning.* One species, the *Bacterium coli,* is frequently used as an index for the determination of the presence of sewage in water.

Pasteurella.—The organisms of this genus are all rod-shaped cells, Gram-negative, and show bipolar staining. They have very slight powers of fermentation. Members of this genus produce many diseases in man and animals, including the *bubonic plague* of man and the *hemorrhagic septicemias* of animals, such as fowl cholera and related diseases in swine, sheep, cattle and horses.

Hemophilus.—The organisms belonging to this genus are minute, parasitic, Gram-negative rods, which grow in the

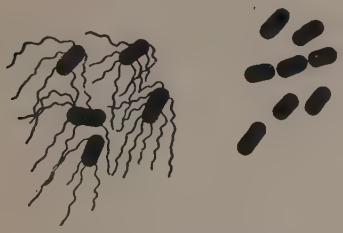

FIG. 24.—BACTERIUM.

FIG. 25.—PASTEURELLA

laboratory only in special media, preferably containing hemoglobin. The most important species is *Hemophilus influenzae,* the organism which has been supposed to cause influenza and which is quite certainly associated with many cases of the disease.

Lactobacillus.—The cells are rod-shaped, often long and relatively slender, Gram-positive, and nonmotile. Some species have very short cells and are almost coccuslike. No endospores are developed. Acid, particularly lactic acid, is usually produced in considerable quantities from carbohydrates. Many of the species prefer to grow in the ab-

sence of oxygen, although they are not strictly anaërobic. Included in this genus are many bacteria important in the souring of milk, the formation of lactic acid in silage, sauer kraut, etc.

FIG. 26.—HEMOPHILUS.

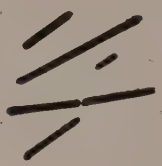

FIG. 27.—LACTOBACILLUS.

VI. FAMILY SPIRILLACEAE

The organisms that belong to this group are curved rods. Two genera are included, namely, *Vibrio* and *Spirillum.*

Vibrio.—This genus includes short curved rods, motile by means of one, two or three polar flagella. Many are intestinal parasites and some are capable of causing disease. The most important organism is the *Vibrio cholerae* producing Asiatic cholera in man.

Spirillum.—Organisms belonging to this group are longer, spiral cells, with a tuft of relatively long flagella at the tip. They are not uncommon in water, and partic-

FIG. 28.—VIBRIO.

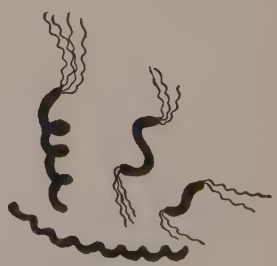

FIG. 29.—SPIRILLUM.

ularly in water that contains decaying organic matter. None of the species are of very much economic importance.

Order Actinomycetales

The organisms belonging to this order usually have the cells somewhat elongated, frequently filamentous, and with a decided tendency to the formation of branches. In some genera a definite branched mycelium is developed. The cells frequently show swelling, or are clubbed or irregular in shape. Endospores are not produced, although conidia are formed by some genera. Many of the genera are Gram-positive and all are nonmotile. Some species are parasitic in animals or in plants. Most of the species are aërobic. The genera belonging to this group are *Actinomyces, Mycobacterium, Corynebacterium,* and *Pfeifferella.*[1]

Actinomyces.—The organisms belonging to this group form fine threads (or mycelium,) which break up into short segments which function as spores or conidia. Some of the species are parasitic, one, the *Actinomyces bovis,* producing lumpy jaw in cattle. Many other species are widely distributed in the soil, apparently growing upon decaying roots and similar organic matter.

Mycobacterium.—These organisms are slender rods which are stained with difficulty, but when once stained are resistant to decolorization by acids, that is, they are termed *acid fast.* The cells are sometimes swollen, showing club shapes or forked forms, occasionally the cells are branched. They are nonmotile and Gram-positive. Some of the species are present in the soil, a few are pathogenic to man and animals. The most important of the diseases caused are tuberculosis and leprosy in man, and paratubercular dysentery in cattle.

[1] Other genera are *Actinobacillus,* one species producing the disease called actinobacillosis in cattle; *Leptotrichia* found in the mouth; *Erysipelothrix* producing disease in man and animals, particularly the disease swine erysipelas; and *Fusiformis,* including certain mouth and throat forms, some of them associated with diseases of the throat and the mouth.

Corynebacterium.—The cells are slender, often slightly curved rods with a tendency toward the formation of clubs, and containing granules which give the cells when stained a barred or irregular appearance. They are not acid fast but are Gram-positive and aërobic. Most species are parasites, the most important being *Corynebacterium diphtheriae*, the cause of diphtheria in man.

Pfeifferella.—These organisms are nonmotile rods, slender, and Gram-negative, staining poorly, sometimes forming threads, and showing a tendency toward branching. When grown upon potato in the laboratory they develop a characteristic honeylike growth. The most important species is *Pfeifferella mallei,* the organism which causes the disease glanders in the horse.

Order Spirochaetales

The organisms belonging to this group in many respects resemble the protozoa, and may be regarded as intermediate between the true bacteria and the protozoa. They are all more or less curved rods, frequently very slender. Many species are motile but there has been no definite demonstration of flagella. They multiply either by longitudinal or transverse fission. It is probable that in some cases they have a relatively complex life history . Several genera have been described, the most important being *Treponema.*

Treponema.—The organisms belonging to this genus are exceedingly slender spiral rods, motile by means of flexuous bending of the body. The most important species is *Treponema pallida,* the cause of the disease syphilis in man.

CHAPTER IV

MORPHOLOGY AND CLASSIFICATION OF THE YEASTS

Size, Shape, and Grouping of Yeast Cells.—Yeasts are usually somewhat larger than bacteria. This usually renders comparatively easy differentiation from the bacteria. The yeast cell is usually spherical, ovoid, or ellipsoid in shape, occasionally it is considerably elongated or even cylindrical. In most species there is very little of the regularity of grouping which is characteristic of many types of bacteria. The cells occur in masses or occasionally in chains or filaments. Yeasts are typically unicellular plants, and can by this means be differentiated from the molds, although in a few species the chains of cells (filaments) approach in appearance the hyphae of the true mold.

Structure

The cell wall is relatively thin in young yeast cells but may be considerably thickened in old. Occasionally the cells are embedded in gelatinous masses. The chemical composition of the cell wall has not been clearly established. It is frequently said to consist of *yeast cellulose,* although not identical in composition with the cellulose of the cell walls of higher plants. The organism practically never produces definite capsules and is never motile, that is, flagella are not produced.

Cell Contents.—The contents of the cell are somewhat more clearly differentiated than is the case with the bacteria. Furthermore, one may note marked differences in the appearance of cell contents of young cells and older

cells. The young cell has a very thin cell wall, protoplasm which is relatively homogenous, and a relatively large nucleus. The latter is easily demonstrated by the use of appropriate stains. As the cells increase in size, vacuoles begin to appear in the protoplasm; both in these vacuoles and in the protoplasm are formed granules which stain intensely with methylene blue, the so-called *metachromatic granules*. Eventually still larger vacuoles develop, which in some cases are crowded full of granules giving the yellow *glycogen* reaction with iodine. Still later the glycogen may disappear but the relatively large vacuoles persist. In an old cell the cytoplasm and nucleus may constitute a relatively small proportion of the cell contents.

By means of suitable reagents it is possible to demon-

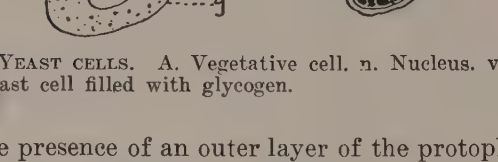

FIG. 30.—YEAST CELLS. A. Vegetative cell. n. Nucleus. v. Vacuole. B. Yeast cell filled with glycogen.

strate the presence of an outer layer of the protoplasm, the *ectoplast*, corresponding in function to that found within the bacteria. It undoubtedly acts as a semipermeable membrane in the determination of substances which may leave and enter the cell.

Vegetative Multiplication of Yeast Cells.—Most of the common yeasts multiply vegetatively by a process of budding. A few species belonging to a single genus (*Schizosaccharomyces*) multiply by fission, in a manner analogous to that already described for bacteria.

Those yeasts which multiply by budding show first a minute protuberance on one side of the cell. The nucleus of the mother cell goes to a point near the opening between

the cell and the bud, elongates in the direction of this opening and eventually pinches off a nucleus which migrates into the bud. This rapidly increases in size and the opening between the two cells soon closes. In a very young bud the cell wall is either absent or very thin, but soon

FIG. 31.—MULTIPLICATION OF YEASTS BY BUDDING.

develops, probably in all cases becoming evident before separation from the mother cell occurs.

Development of Spores.—All species of true yeasts are capable of producing spores under suitable conditions. These conditions differ with the species. In most species the spores are not developed in the ordinary cultural media or by the usual methods. With many of the more common yeasts of economic importance sporulation may be induced by placing young vigorous cells under such conditions that they will have an abundance of oxygen, plenty of moisture, and the right temperature. This is most frequently accom-

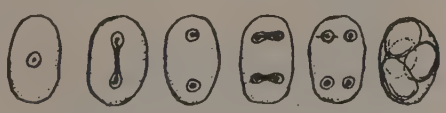

FIG. 32.—DEVELOPMENT OF SPORES IN YEAST.

plished by preparing small, flat gypsum blocks (plaster of Paris). These are placed in deep Petri dishes, water

poured into the Petri dish until it reaches half the height of the block, when the yeast mass to be studied is spread over the surface. This is placed in a thermostat at the right temperature, usually about 25° C. The cells will usually develop spores in from twenty-four to forty-eight hours. Some species of yeast which form films over the surface of their nutrient solutions may develop the spores in such films.

Yeast spores, like the spores of bacteria, are always formed inside the mother cell, but differ in that usually more than one spore is formed within a single cell.

The first evidence of sporulation in yeasts is the division of the nucleus first into two, then into four, six, or eight nuclei, by a process of mitosis. Each one of the nuclei then surrounds itself by a bit of protoplasm and finally by a cell wall or membrane. The spores grow in size by absorption of the cytoplasm from the mother cell until they come to occupy practically the entire interior. The number of divisions of the nucleus determines the number of spores which will develop. This is usually fairly constant in a given species, although variations may be found. Yeasts are known which typically produce one, two, four and eight spores to a cell.

The mother cell with its spores shows such close relationship to the spore-bearing sacs produced by certain of the fungi that it is usually given the name *ascus,* and the spores are known as *ascospores.*

The spores vary not only in number but in shape and size as well. Some are spherical, some elliptical, others are hat-shaped, or even elongate. Some have a single membrane and others apparently a double membrane.

Sexual reproduction of a primitive type may be observed in some species of yeasts, though not with the most common types. In a few species cells which are about to form spores fuse in pairs, the nuclei also fuse and then divide

to form the number of nuclei characteristic of the species, each nucleus becoming a spore. In some forms the cells which fuse are of the same size; in others they differ. In one the spores fuse in pairs during the process of germination and the young yeast cells are produced by budding.

Occasionally yeast cells surround themselves with an unusually heavy membrane and pass into a resting state. Such cells are termed *chlamydospores.*

The Classification of Yeasts

Relationships of True Yeasts.—The development of asci and ascospores by yeasts show that their relationship is primarily to the group of fungi termed ascus-fungi or *Ascomycetes.* Yeasts are sometimes conveniently separated into *true yeasts* and *false yeasts.* The former are those which have already been described. To the latter belong certain forms which have every appearance of being the vegetative stages of true yeasts, but which do not produce spores, and are frequently not active in causing alcoholic fermentation. An organism of the latter type is sometimes termed a *torula.*

Classification of the Yeasts.—Botanists recognize a dozen or more different genera of yeasts. Only two are of sufficient importance to be considered here. The first of these is the genus *Saccharomyces,* which includes those common yeasts which multiply by process of budding, and *Schizosaccharomyces,* in which the cells multiply by a process of fission.

Saccharomyces.—To this genus belong most of the yeasts of economic importance, including those which are used in wine making, in manufacture of beer and commercial alcohol, and in bread making. Many different species have been described and several different methods of classification have been used.

One common method of differentiating yeasts is based

upon the type of fermentation. Some yeasts settle relatively rapidly to the bottom of the fermenting liquid and do not form a heavy scum or pellicle over the surface. They are termed *bottom yeasts.* Certain of the wine and cider yeasts are of this type. In other species growth is usually characterized by the development of a foamy scum on the surface. Such are termed *top yeasts.* Intermediate forms, however, are known.

Many of the great brewing districts in Europe have developed special strains of yeasts which are used for manufacture of particular types of beverages. Such yeasts have been named after the town, locality, or establishment in which they originated. For example, one may read of Saaz yeasts, of Carlsberg bottom yeasts, etc.

Among the earlier investigators an attempt was made to separate the various species of yeasts on the basis of shape. The ordinary brewers' or bread yeast, known as *Saccharomyces cerevisiae,* was described as oval or nearly spherical; wine yeasts were found usually to be ellipsoid or elongate, and were termed *Saccharomyces ellipsoideus.* The name *Saccharomyces pastorianus* was given to those which produced long or cylindric cells. The conditions under which yeasts are grown, however, influence to such a marked degree the shape and size of the cells that this method of differentiation is frequently difficult to use.

The most satisfactory methods for differentiation of yeast species are based upon such morphological differences as may be observed microscopically, the development of spores, the ability of yeasts to ferment particular sugars, to grow in the presence or absence of air, and to produce definite quantities of alcohol. It is customary to recognize six groups of yeasts, the groupings being determined by the carbohydrates which may be fermented by the formation of alcohol and carbon dioxide. The following table gives the characteristics of these groups:

Number of group	Production of Alcohol and Gas from			
	Dextrose	Saccharose	Maltose	Lactose
1	+	+	+	—
2	+	+	—	—
3	+	—	+	—
4	+	—	—	—
5	+			+
6	—	—	—	—

Schizosaccharomyces.—As was noted above, the organisms belonging to this genus multiply by fission rather than by budding. The cells when about to produce spores fuse in pairs and within the resulting cell eight spores are usually developed. Several species have been described. One is the active fermentative agent in the production of an African beverage made from millet.

CHAPTER V

MORPHOLOGY AND CLASSIFICATION OF THE MOLDS

STRUCTURALLY the molds differ from the bacteria and the yeasts in that they are multicellular, that is, the plant body is more complex than that of bacteria or yeasts, and is made up of numerous cells. Furthermore, the cells which make up the plant body of a mold are not all uniform in size, shape or function. Some are differentiated for purposes of securing nutrients, others may be differentiated for purposes of reproduction.

It should be emphasized that the molds as considered here do not constitute a group recognized as such by the botanist. All belong to the great class Fungi, but they are scattered among several distinct subgroups.

The plant body of the mold is made up of a mass of threads, usually branched. This whole mass of threads taken collectively, that is, the entire plant body, is termed the *mycelium*. The individual threads are called *hyphae*. In most molds the hyphae are of two principal types, those which are differentiated for the purpose of producing spores, called the *fertile hyphae,* and those which serve to secure nutrients for growth, termed the *vegetative hyphae*.

Structure of Mold Hyphae.—Two kinds of hyphae may be recognized among the molds. In the first the cells of the hyphae are separated from each other by definite cell walls, that is, the threads consist of definite cells arranged in chains, that is, cross walls are formed in the hyphae. A cross wall is termed a *septum,* and the hyphae are said to be *septate*. In the second group of molds the hyphae do not usually show cross walls except during the process of

sporulation. The hyphae are more or less branched, continuous tubes. Such a mycelium is said to be *nonseptate.*

In general those molds which show a separate mycelium have a single nucleus with the cytoplasm in each section of the hyphae. In those molds which are nonseptate numerous nuclei are to be found scattered throughout the protoplasm. A cell is sometimes defined as a nucleus surrounded by a bit of protoplasm, hence it is not incorrect to speak of the nonseptate molds as being multicellular.

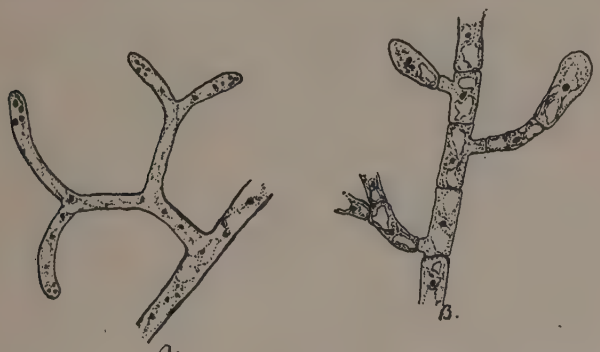

FIG. 33.—TYPES OF HYPHAE IN MOLDS. a. Nonseptate hypha. Note numerous nuclei and absence of cross walls or septa. b. Septate hypha. Note single nucleus in each cell.

Structure of the Mold Cell.—The cell of the mold does not differ in many respects from that of the yeast. The cell wall consists in some instances of a substance closely resembling cellulose, in others it may contain callose and pectose. It is sometimes markedly thickened. This is particularly true of the outer walls of spores and of resting cells. Such are sometimes cutinized. Such cells may occasionally show ridges, thickenings, spines, knobs, or other protuberances.

The nuclei may ordinarily be readily differentiated from the remainder of the cytoplasm. In the young cell the cytoplasm is relatively dense. In the older cells it becomes

vacuolated, and in cells which have reached maturity frequently the protoplasm containing the nucleus occupies but a small portion of the cell volume, being reduced to a comparatively thin layer just inside the cell wall. Granules of various kinds, including metachromatic granules, globules of fat and of glycogen may develop. Apparently these products may function as reserve food materials.

Mold Growth.—Most molds increase in size by growth at the tip of the hyphae. Any cells lying back of the tip that start to grow develop into branches. Such growth is termed *apical.* A few molds are known in which all cells may continue to grow in length and divide. Such growth is termed *intercalary.* In apical growth the tip cell only divides, while in intercalary growth cells throughout the filament may divide.

Multiplication in Molds.—Practically all molds multiply by means of specialized cells termed spores. In many instances these spores are numerous and well adapted to carry the organism over unfavorable conditions and to facilitate its distribution.

A single species of mold may produce several kinds of spores. A few molds have relatively complex life histories, producing a different type of spore at each stage in development. In general the spores developed belong to two types, those which are *asexual* in origin and those which are *sexual.* The *asexual* spore may be defined as one which is not the result of the fusion of two sex cells or *gametes.*

Practically all of the common types of molds produce asexual spores, some several kinds. A smaller number of molds produce also sexual spores. From the standpoint of the botanist who is seeking to determine true interrelationships, the sexual spores are of great interest. From the standpoint, however, of the practical worker it is possible to differentiate the molds satisfactorily by observing the types of asexual spores produced.

Sexual Reproduction in Molds.—Molds having nonseptate mycelium produce a type of sexual spore termed a *zygospore*. Those molds with septate mycelium, which produce sexual spores, develop *ascospores*.

The process of zygospore formation can most readily be understood by reference to the accompanying figure. Two filaments lying near each other send out branches which approach each other and finally touch. The cell walls separating the two tips are absorbed, resulting in union of the protoplasm of the two cells. Cell walls are then formed, separating the fused mass of protoplasm from the parent hyphae. The nuclei of the two masses of proto-

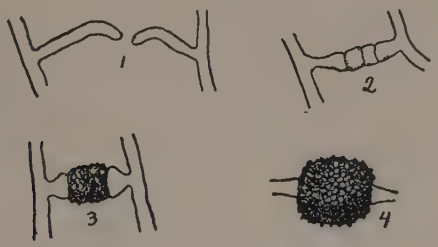

FIG. 34.—DEVELOPMENT OF A ZYGOSPORE IN A MUCOR.

plasm apparently fuse in pairs. The resultant spore or cell enlarges markedly in size, develops a very thick, usually brownish cell wall, frequently marked by spines or by irregular thickenings. Under favorable conditions this zygospore germinates and develops a new mold.

The development of ascospores is somewhat more complex than that of zygospores. Two cells, frequently of somewhat different size and shape, develop from the same or adjoining threads. The cells coil together and the tips of the cells fuse. The cell resulting from this fusion begins to grow, branching more or less, and becomes surrounded in most cases by a mass of hyphae, which develop from adjacent hyphal threads. The branches of the fertilized

cell develop into sacs. In each one of these sacs, or asci, two or more spores, usually eight, form. The structure developed surrounding the spore sacs is termed a *perithecium.* This is usually globular in shape and frequently brightly colored. Comparatively few of the molds usually encountered in the laboratory produce these perithecia. An exception is to be found in certain common species of *Aspergillus,* which readily develop orange or bright yellow perithecia under suitable conditions.

Asexual Reproduction in Molds.—The asexual spores of molds may be developed free or they may be enclosed in special spore cases termed sporangia. Many of the molds

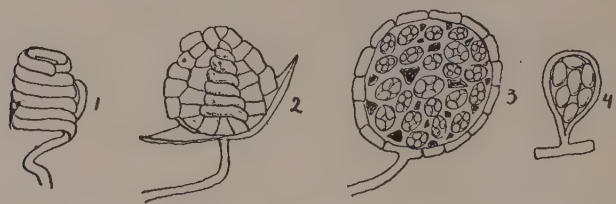

FIG. 35.—FORMATION OF ASCOSPORES AND ASCI IN ASPERILLUS. 1, 2, 3. Stages in the development of a perithecium. 4. A single ascus from interior of the perithecium. (Adapted from De Bary.)

with nonseptate hyphae produce these sporangia, but they are never developed by the molds with septate hyphae.

Sporangium Formation.—The development of the sporangium may be illustrated by the genus *Mucor.* The first evidence of spore formation is the appearance of a fertile hypha produced at right angles to the surface on which the mold is growing. This becomes enlarged at the end, increases in length, and finally consists of a relatively long thread with a much swollen tip. The interior of the latter is filled with protoplasm containing numerous nuclei. A special wall then forms extending from the tip of the thread up into the swollen apex. The nuclei surround themselves by bits of protoplasm and develop around these, in turn,

definite cell walls forming spores. The spore case is termed a *sporangium.* The structure reaching to the interior is a *columella,* and the stalk upon which the sporangium is borne a *sporangiophore.* In some species the sporangiophore may branch, each branch bearing a terminal sporangium. When ripe the sporangium walls break and spores are scattered.

Conidia.—Asexual spores of molds not bearing sporangia are frequently termed *conidia.* Asexual spores may be developed on specialized branches or by changes occurring in the vegetative cells of the mycelium.

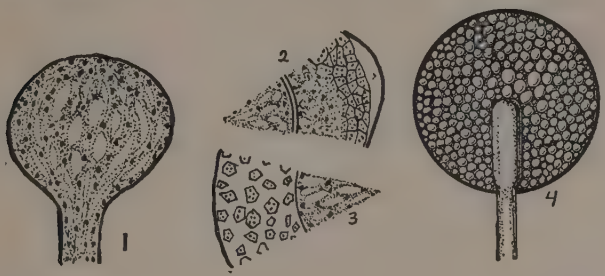

FIG. 36.—DEVELOPMENT OF SPORES AND SPORANGIA OF MUCOR. 1. Young sporangium with numerous nuclei. 2. Segmentation of protoplasm. 3. Segmentation of protoplasm and separation of spores. 4. Mature sporangium. (Adapted from Blakeslee.)

Chlamydospores are asexual spores having a relatively thick cell wall. *Oïdia* are developed when the vegetative mycelium breaks up into spores without any differentiation of special spore-bearing threads. In most molds a definite fertile thread termed the *conidiophore* is developed to produce the conidia. The conidiophore may be of many shapes and types, sometimes simple, sometimes branched, and sometimes club-shaped.

Conidia borne on the tip of branches may be developed in one of two ways. In some species of molds the tip of the conidiophore pinches off spores one after another; if they stick together a chain is formed in which the spore

nearest the mother cell is the youngest. In other molds spore formation results from the budding out of a cell (not a pinching off) from the terminal cell. The spore then grows to its full size, and from its tip another spore buds out. A continuation of this process also results in the formation of a chain of spores. In this type of spore formation, however, the chains are sometimes branched, that is,

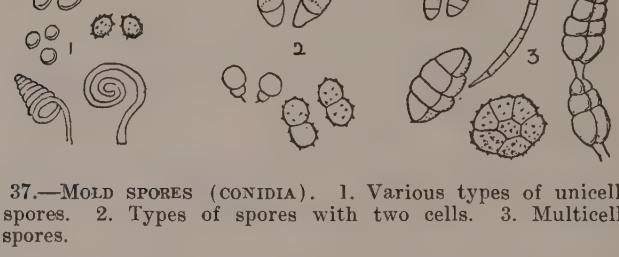

FIG. 37.—MOLD SPORES (CONIDIA). 1. Various types of unicellular spores. 2. Types of spores with two cells. 3. Multicellular spores.

more than one spore may bud from a single cell. The terminal cell of the chain is always the youngest.

The conidia developed by molds are of many shapes and

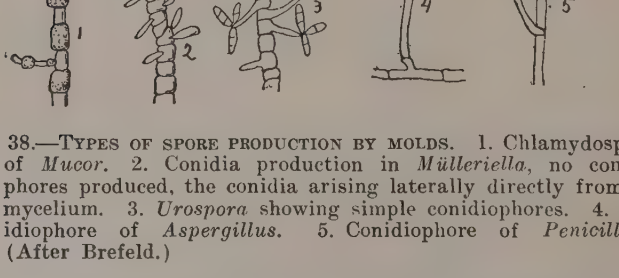

FIG. 38.—TYPES OF SPORE PRODUCTION BY MOLDS. 1. Chlamydospores of *Mucor*. 2. Conidia production in *Mülleriella*, no conidiophores produced, the conidia arising laterally directly from the mycelium. 3. *Urospora* showing simple conidiophores. 4. Conidiophore of *Aspergillus*. 5. Conidiophore of *Penicillium*. (After Brefeld.)

sizes. The simplest type of conidium is that which is single-celled and oval or spherical in shape. Other single-

celled spores may be elongate or even spindle-shaped, cylindrical, or club-shaped. Two-celled spores occur in many species of molds. Others produce elongate spores, having several or many cross walls. The spores of some molds are many-celled, the walls being not only transverse but longitudinal or irregularly disposed. The spores of some molds branch, in others they are curved, sometimes being coiled like a spring.

Spores are usually adapted to wind distribution, being caught up by air currents and carried to considerable distances. They are, therefore, commonly present in the air and are constantly observed in the laboratory. When they are brought under favorable conditions for growth, they germinate, developing a mycelium.

Classification of the Molds

It has already been noted that the term mold as used in bacteriology is not recognized in this exact sense by the botanist. The molds are plants belonging to several different groups of fungi. They are all alike, however, in possessing a much-branched mycelium and growing as a velvety or cottony mass upon ordinary culture media, or upon decaying materials.

Many different kinds of molds are known, in fact, more than a hundred different genera have been described. Of these, however, comparatively few are common, and the student should readily recognize nine tenths of the molds with which he may come in contact by comparatively simple microscopic examination. In many cases the genus to which the mold belongs can be determined by its general appearance. Some of these common genera have many species. In the following account a description will be given of the commonest of the genera together with notes on methods of differentiating some of the species.

The following key based upon asexual spore production differentiates nine genera of molds:

KEY FOR DIFFERENTIATION OF COMMON GENERA OF MOLDS

A. Spores borne in sporangia. Mycelium non-septate.
  B. Sporangiophores arising in clusters from the nodes of runners or stolons. ..... 1. *Rhizopus.*
  BB. Sporangiophores arising singly, without stolons. ..... 2. *Mucor.*
AA. Spores (conidia) never borne in sporangia.
  B. Neither conidia nor hyphae smoky or dark in color.
    C. Conidia one-celled.
      D. No distinct conidiophores. Conidia formed as oïdia..... 3. *Oöspora.*
      DD. Conidia on distinct conidiophores.
        E. Conidiophores enlarged or club-shaped at tip. ..... 4. *Aspergillus.*
        EE. Conidiophores branched at tip. .... 5. *Penicillium.*
    CC. Conidia two-celled, pear-shaped. .... 6. *Trichothecium.*
  BB. Either conidia or hyphae smoky or dark, or both.
    C. Conidia one-celled, in branched chain. .. 7. *Hormodendrum.*
    CC. Conidia more than one-celled.
      D. Like *Hormodendrum,* but condidia two-celled when old. ..... 8. *Cladosporium.*
      DD. Spores many-celled, club-shaped, in chains. ..... 9. *Alternaria.*

Rhizopus.—Molds belonging to this genus are frequently termed the black or bread molds. They are among the most common, occurring upon bread, decaying vegetables, and similar material. When young they are cottony and white, as they grow older they become grayish because of the development of numerous dark sporangia. The vegetative mycelium spreads through the medium upon which the organism is growing. When ready to produce spores, long slender threads are thrown out which grow until the tip touches some solid material. For example, in the laboratory the tip may touch the glass side of the dish in which the organism is growing. From this tip are sent out clusters of dark brown, short branches, termed *rhizoids.* These act as holdfasts, they are not roots in any sense. The

hypha, called a stolon, grows on from this cluster of rhizoids until it again attaches itself at some other point. From each cluster of rhizoids there arises a group of sporangiophores which grow into the air, developing at the tip the large dark brown or black sporangia. These sporangia are readily recognized under the microscope. When mounted in a drop of water the ripe sporangium usually collapses, the outer wall disappearing and the spores go free. The columella in this form is partly adherent at its base to the outer wall of the sporangium. Usually when the sporangium collapses it also collapses, the lower side invaginates,

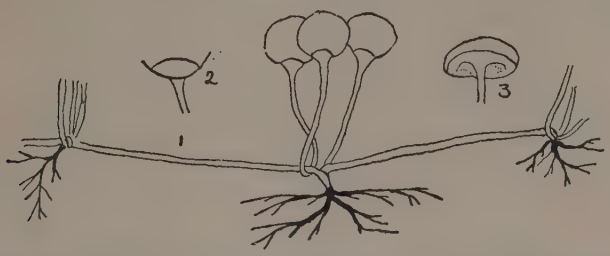

FIG. 39.—RHIZOPUS. 1. Growth habit of the mold, showing stolons, rhizoids, sporangiophores and sporangia. 2, 3. Columella.

and together with the stalk or sporangiophore gives rise to a structure having the appearance of a small toadstool or umbrella.

Some species of *Rhizopus* are used commercially in the transformation of starchy materials into sugar, precedent to the fermentation of the sugar into alcohol in the commercial manufacture of this material.

The most common of the species of *Rhizopus* is *Rhizopus nigricans,* the common black bread mold. The species used in hydrolysis of starch are *Rhizopus oryzae* and *R. japonicus.*

Mucor.—The genus *Mucor* is not as abundant as *Rhizopus* but is found under the same general conditions. Usually the mold is not quite so coarse. It does not develop stolons.

Its sporangiophores are sometimes branched, do not arise in clusters, and no rhizoids are developed. In the sporangium the columella is not attached to the sporangium wall, but may be spherical in shape, pear-shaped, and in one species pointed or covered with spines. The various species of

FIG. 40.—COMMON SPECIES OF MUCOR. a. Sporangiophore showing branching and grouping of sporangia. b. Longitudinal section through sporangium. c. Spores. d. Columella. 1. *Mucor hiemalis.* 2. *Mucor piriformis.* 3. *Mucor mucedo.* 4. *Mucor plumbeus.* 5 *Mucor alternans.* (1, 2, 3, after Wehmer. 4, 5, after Gray.)

Mucor are separated from each other on the basis of the size, shape, and color of the sporangium, size and shape of columella, and the branching of the sporangiophores.

Some eleven species of *Mucor* have been described and are comparatively common. They may be differentiated from each other by use of the key given in the footnote.[1]

[1] KEY TO SPECIES OF MUCOR

A. Sporangiophores rarely or never branched.
  B. Columella spherical ..... *Mucor hiemalis.*
  BB. Columella pear-shaped (piriform) ..... *M. piriformis.*
  BBB. Columella elongate to ellipsoidal ..... *M. mucedo.*
AA. Sporangiophores usually branched.
  B. Columella usually knobbed or spiny near tip.. *M. plumbeus.*
  BB. Columella not roughened at tip.
    C. Rarely fruiting at room temperatures, important in commercial alcohol manufacture, rapidly saccharifying starch ..... *M. rouxii.*
    CC. Readily fruiting at room temperature, not commercial types.
      D. Sporangiophores with definite main stem and secondary lateral branches, racemose.
        E. Columella ovoid ..... *M. racemosus.*
        EE. Columella spherical

One of the species of *Mucor, Mucor rouxii,* like the species of *Rhizopus* above mentioned, has been used in the saccharification of starch. Some of the species produce heavy-walled resting cells or chlamydospores in the vegetative mycelium. This is particularly true of *Mucor racemosus.*

Oöspora.—This genus is sometimes known as *Oïdium.* The most common species is *Oöspora lactis,* a mold very common upon sour milk and cheese; in fact, it is to this mold that many types of cheese owe in part their characteristic flavor and aroma. The mycelium of this fungus grows very largely below the surface of the medium on which it is developing, and later splits up by transverse fission into numerous spores or oïdia. These spores frequently do not stand out upon the surface of the medium but are developed below. No distinct conidiophores are formed. The mycelium shows a characteristic type of branching. Instead of having main shoots with side branches, the terminal cells usually divide and two branches of equal size develop from tip. This type of branching is termed *dichotomous.*

Aspergillus.—The spores of *Aspergillus* are relatively common in the air. The organism grows on all sorts of decaying vegetation, on moldy corn, grain, bread, etc. In color it is usually greenish, yellow, orange, black or brown. The mold has generally a velvety or powdery appearance. The hyphae are much branched and septate. The fertile hyphae grow out into the air, the tip becoming enlarged or club-shaped. From this enlarged tip are developed numerous small branches called *sterigmata.* These usually

F. Sporangium gray, yellow..... *M. erectus.*
FF. Sporangium black..... *M. fragilis.*
DD. Branches of sporangiophore nearly equal, cymose.
E. Sporangia borne irregularly..... *M. ambiguus.*
EE. Sporangia in two rows, alternating.
F. Spores spherical to short ellipsoidal ..... *M. circinelloides.*
FF. Spores longer, ellipsoidal..... *M. alternans.*

cover the entire end and frequently the entire swollen portion of the conidiophore, giving it something of the appearance of a war club covered with spikes. These sterigmata in some species branch into groups of branches, and in other species they give rise directly to the spores. The spores are pinched off in chains from the tips of the primary or secondary sterigmata. In some species the spores radiate in all directions from the head, in others the chains cling together giving rise to a long column of spores like a plume.

Many species of *Aspergillus* have been described. A few of them are of considerable economic importance. The

FIG. 41.—SPECIES OF ASPERGILLUS. a. View of conidiophore and condia b. Longitudinal section through conidiophore to show attachment and arrangement of sterigmata and conidia. c. Conidia. 1. *Aspergillus fumigatus.* 2. *A. niger.* 3. *A. glaucus.* (Adapted from Wehmer.)

species known as *Aspergillus fumigatus*, for example, may produce a type of pneumonia in birds when it is inhaled, and it may also be pathogenic for other animals. *Aspergillus niger* has been much studied because of its ability to produce a great variety of fermentative changes. The commonest is probably *Aspergillus glaucus*, a green species not uncommon on canned fruits, jams, moldy grains, silage, and in similar locations. It frequently produces abundant yellow perithecia containing asci and ascospores. *Aspergillus oryzae* has been used in the manufacture of industrial alcohol, for the conversion of starchy material into sugar preliminary to the alcoholic fermentation.

The key given in the footnote will enable the student to separate some of the more common species.[1]

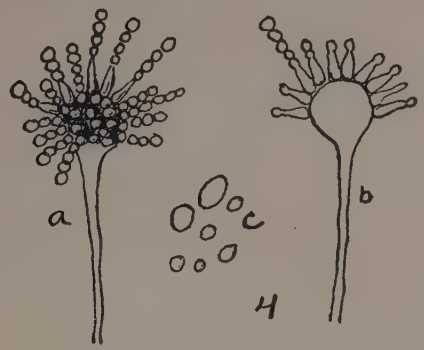

FIG. 42.—ASPERGILLUS ORYZAE.

[1] KEY TO ASPERGILLUS SPECIES

A. Spores white or nearly so.
  B. With unbranched sterigmata..... *Aspergillus candidus.*
  BB. With branched sterigmata..... *A. albus.*
AA. Spores colored.
  B. Spores green, grayish, bluish or yellowish-green.
    C. With unbranched sterigmata.
      D. Producing perithecia readily.
        E. Perithecium naked, not embedded. *A. glaucus.*
        EE. Perithecium embedded.
          F. Tip of conidiophores only slightly swollen, club-shaped, sterigmata along sides for a considerable distance. ..... *A. clavatus.*
          FF. Tip of conidiophore hemispherical, sterigmata produced only from terminal portion..... *A. fumigatus.*
      DD. Not producing perithecia (so far as known).
        E. Tip of conidiophore, very large, elongates 80-100×500-800μ..... *A. giganteus.*
        EE. Tip of conidiophore smaller. spherical, or hemispherical.
          F. Conidiophore rough, warty.... *A. flavus.*
          FF. Conidiophore smoother..... *A. oryzae.*
    CC. With branched sterigmata.
      D. Mycelium rusty brown..... *A. versicolor.*
      DD. Mycelium not so.

Penicillium.—Certain species of this genus probably are the most common of all molds. They are variously colored, usually grayish-green or bluish-green. They occur upon the surfaces of decaying vegetable matter such as silage, oranges, lemons, bread, jams, jellies, etc. The spores are relatively common in the air and are usually met with in the laboratory. In many respects these organisms resemble *Aspergilli.* They differ primarily in the manner in which the spores are produced. The erect conidiophores branch at the tip, a group of two, three, or four branches usually being formed. These continue parallel to each other and usually again branch. This may occur a second or a third time. The terminal branches pinch off chains of spores which may reach a considerable length. Microscopically the conidiophore with its branches, tip and long chains of spores resembles a brush, hence the name *Penicillium* (a little brush).

FIG. 43.—PENICILLIUM.

Certain of the species of *Penicillium* are of importance in the ripening of cheese, particularly Roquefort, in which *Penicillium roquefortii* is present, and Camembert, in which *Penicillium camembertii* occurs. The char-

E. Tip of conidiophore club-shaped, sterigmata both lateral and terminal ..... *A. pseudoclavatus.*
EE. Tip of conidiophore hemispherical, sterigmata terminal..... *A. nidulans.*
BB. Spores black or dark brown.
C. With unbranched sterigmata..... *A. calyptratus.*
CC. With branched sterigmata..... *A. niger.*
BBB. Spores, yellowish-brown, yellow, brown, or reddish.
D. With unbranched sterigmata, coffee-brown spores..... *A. wentii.*
DD. With branched sterigmata, yellow-brown spores..... *A. ochraceus.*

acteristic flavor and consistency of these cheeses is due in large measure to the growth of these *Penicillia.*

Some hundreds of species of *Penicillium* have been described. They are comparatively difficult to identify because of the variation in the appearance of the same mold

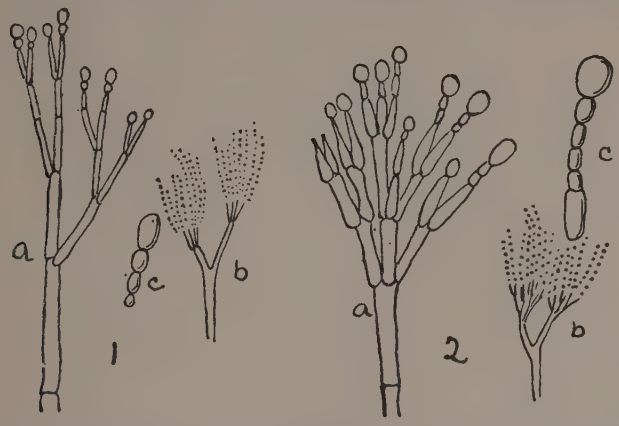

FIG. 44.—PENICILLIUM. 1. *Penicillium roquefortii.* 2. *Penicillium camembertii.*

when grown under different conditions. A few species may frequently be determined from the food or substratum on which they are growing. The following key to such species has been adapted from Thom.[1]

[1] U. S. Dept. Agriculture, Bureau of Animal Industry, Bull. 118.

KEY TO SOME COMMON SPECIES OF PENICILLIUM

A. Growing on cheese.
  B. Camembert or Brie.
    C. Floccose colonies, white to gray-green ..... *Penicillium camembertii.*
    CC. Powdery colonies, yellowish-white. ..... *P. brevicaule var. glabrum.*
    CCC. Forming yellow-brown areas, spores rough..... *P. brevicaule.*
  BB. Roquefort, forming green streaks inside cheese..... *P. roquefortii.*
AA. Growing on citrus fruits (lemons, oranges).
  B. Mold colonies blue-green..... *P. italicum.*
  BB. Mold colonies olive-green..... *P. digitatum.*

Trichothecium.—A common pink mold occurring particularly on decaying fruit such as apples is *Trichothecium roseum.* In some cases it may cause considerable damage due to the development of rot. The spores are not uncommon and sometimes the mold makes its appearance on laboratory media. The mold itself is white in color, sends up numerous unbranched conidiophores which produce a cluster of two-celled pear-shaped spores at the tip. The spores do not occur in chains. The terminal cell of the spore is larger than the basal cell.

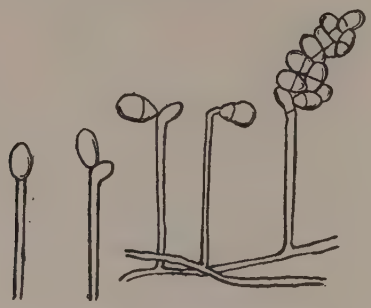

FIG. 45.—TRICHOTHECIUM.

Hormodendrum and Cladosporium.—These molds are abundant upon decaying paper, straw, and similar materials. They usually produce irregular dark or sooty patches of various sizes. The mycelium and spores are both smoky or fuscous in color. The conidiophores are usually well differentiated, branching more or less at the tip and producing chains of spores which likewise branch. These two molds belong to the group in which the terminal spore is the youngest in the chain. The most common species is the *Cladosporium herbarum.* The mycelium frequently produces many cells of irregular shape and size. The coni-

AAA. Growing on pomaceous fruits (apples, pears). Blue-green colonies forming coremia..... *P. expansum.*

dia of *Hormodendrum* are one-celled, while those of *Cladosporium*, at least in the old and mature colonies, are in part two-celled.

Alternaria.—In this genus the spores are large and club-shaped, occurring in chains, the youngest spore at the tip.

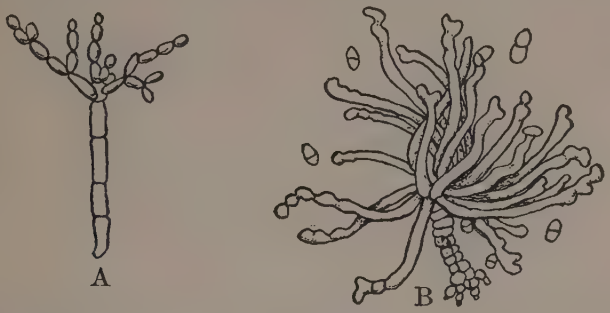

FIG. 46.—HORMODENDRUM AND CLADOSPORIUM. A. *Hormodendrum*. B. *Cladosporium*.

The mycelium is usually also brownish or fuscous in color. The spores are many-celled and muriform. The species *Alternaria tenuis* is abundant in moldy grains, in the soil, and the cells are frequently found in laboratory air.

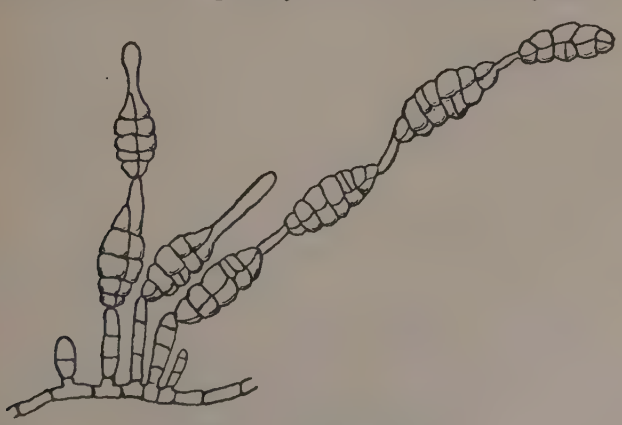

FIG. 47.—ALTERNARIA.

SECTION II

METHODS OF STUDY

CHAPTER VI

CULTURAL METHODS

Whenever practicable it is customary to study cultures of bacteria when grown under laboratory conditions. Many species of bacteria, as they occur in nature, are usually found growing together, that is, in *mixed* cultures. They are widely distributed, making it somewhat difficult to maintain the bacteria in pure cultures after they are once obtained. For an adequate laboratory study of cultural characters it is necessary first to sterilize suitable containers, usually glassware, second to prepare a suitable medium or nutrient mixture upon which the organisms will grow, third to secure the organism to be studied in *pure* culture, that is, free from admixture with all other kinds of bacteria, and fourth to study the characteristic growth and appearance upon the culture media.

Methods of Sterilization

Sterilization may be defined as that process whereby materials may be rendered entirely free from living microörganisms. Bacteria are so widely distributed in nature that it is usually taken for granted that they are present in or upon practically all the materials which are used in the laboratory. Practically the only source of material *naturally* sterile is from the normal tissues of animals or plants. It is, for example, possible so to bleed an animal as to secure sterile blood serum without any process of sterilization. In most cases some artificial method of removing the bacteria is used.

Organisms may be removed or destroyed, that is, materials may be sterilized, either by physical or by chemical means. The process of chemical sterilization will be discussed later under the heading of action of disinfectants and antiseptics. The physical processes of sterilization are the ones most frequently used in preparation of material for the growth of microörganisms. The physical agencies most important in sterilization are heat, light, and filtration.

Sterilization by Heat.—Bacteria, yeasts, and molds, like all higher forms of life, are killed if they are subjected to a temperature sufficiently high, and for a sufficient length of time. To sterilize by heat implies subjecting the material to be sterilized to such a temperature and for such a period of time as will quite certainly destroy all microörganisms which are present. The methods commonly used are exposure to the *direct flame*, to *hot air*, to *streaming steam*, and to *steam under pressure*.

Platinum needles and other small metallic (or occasionally glass) objects used in the laboratory are usually sterilized by placing them directly in the Bunsen flame. A needle, for example, may be quickly raised to a red or white heat. Momentary exposure, under these conditions will, of course, destroy all organisms which are present. Incineration is also the method most commonly used in the disposal of the bodies of animals that have died of infectious diseases, and for the destruction of materials of little value which have been contaminated with disease-producing microörganisms.

Dry heat is commonly used for sterilization of the laboratory glassware such as test tubes, flasks, and pipettes. The so-called hot air oven is ordinarily used for this purpose. Materials to be sterilized are subjected to a temperature of 165° to 175° C. for an hour or more. The long exposure is in part necessary to make sure that the heat has penetrated

to all parts of the material to be sterilized. The cotton which is used for closing the openings of bottles and flasks is usually slightly browned by this exposure. Care should be used that the temperature does not reach so high a point that the cotton is charred.

Many objects which will not withstand heating at high temperatures or in a dry atmosphere may be sterilized by long continued or repeated boiling in water, or exposure to streaming steam, that is, to steam at atmospheric pressure. The latter is usually accomplished by the use of an instrument termed an Arnold sterilizer which supplies a continuous flow of live steam to the box in which the materials to be sterilized are placed. In such sterilization it is frequently customary to heat for fifteen minutes on each of two or three consecutive days. In other cases the material is heated for a longer period at one time.

Sterilization is most efficiently effected by the use of steam under pressure. Certain types of media cannot be sterilized in this fashion because they may be decomposed, but most materials used in the laboratory in media, can withstand a temperature of 110° to 122° C. secured by steam pressure of eight to fifteen pounds per square inch. The apparatus commonly used is some form of an autoclave. In the use of the autoclave care should be exercised that all of the air has been driven out by the entering steam before it is tightly closed, otherwise the temperature will not be as high as would be assumed from the reading on the pressure gauge of the instrument. Ten to twenty minutes exposure at fifteen pounds pressure will destroy any living organism. Where large bulk of material is exposed or where the material is of such a nature that penetration of steam is slow a longer period of time must be used.

Sterilization by Light.—Sunlight destroys microörganisms rapidly. The ultra-violet rays of the spectrum are particularly potent. It is possible quite efficiently and sat-

isfactorily to destroy the bacteria present in transparent fluids, such as water, by exposure to the light from some sources having high ultra-violet and violet intensity. This is most effectively accomplished by the use of the Cooper-Hewitt mercury vapor lamp in a quartz tube. Such apparatus has been used on a commercial scale for the purification and sterilization of water.

Sterilization by Filtration.—Experience has shown that the larger bacteria, at least, are held back when liquids containing them are filtered through porcelain filters (bougies, or candles). These filters in many degrees of fineness (porosity) may be purchased upon the market in many shapes and sizes. Filters are particularly adapted to the sterilization of media which would be decomposed or injured by heat. Many of the materials sterilized by filtration are more or less viscous, such as blood serum, and in consequence do not pass through the filter readily. The rapidity of filtration can be greatly increased by exhausting the air from the flask into which the material is being filtered, the air pressure forcing the material through the pores of the filter. Filters have also been devised whereby it is possible to apply pressure directly to the fluid, forcing it through the pores of the filter with comparative rapidity.

Culture Media

The material or substratum upon which an organism is grown is called a *medium* or *substrate*. If the organism is to be successfully cultivated it must contain all of the materials essential to its growth. The various species of bacteria, yeasts, molds, and protozoa show wide differences in the nutrient materials which they require. Some forms require inorganic material only; others require a mixture of inorganic and organic substances; and some will grow only in the presence of the most complex proteins or similar materials, under conditions simulating those which exist in

the animal body. It is apparent, therefore, that media of many different kinds will be found useful in the laboratory.

Several steps are common to the preparation of all kinds of media. First, there must be the right mixture of the necessary nutrients. Second, these must be present in the right concentration in water. Third, certain substances may or may not be added to cause the medium to be solid or liquid as required. Fourth, the reaction must be adjusted when necessary. Fifth, the medium should be placed in suitable containers. Sixth, it should be sterilized.

Nutrient Substances Used in Preparation of Media.—A few microörganisms, some of them of considerable economic importance, do not require any organic material for food, that is, they are able by oxidation of certain inorganic substances to secure the energy necessary for them to build up complex organic substances from inorganic. In preparing media for such organisms, therefore, it is necessary to use certain inorganic salts. In most instances some salt of nitrogen, either ammonia or nitrous acid, is necessary, likewise some source from which the carbon can be secured, usually a carbonate or carbon dioxide. Most organisms are stimulated in growth by the presence of phosphate. As an example of such a simple nutrient medium may be cited one useful in cultivating from the soil the bacteria capable of oxidizing ammonia to nitrous acid. Such a medium contains ammonium sulphate, calcium or magnesium carbonate, usually a little phosphate and traces of certain other salts, but no organic matter.

Most of the common bacteria and those usually cultivated in the laboratory require more or less organic material as food. Some forms will utilize inorganic nitrogen providing they have organic carbon compounds. They will, for example, take nitrogen from ammonia or from nitrates providing carbohydrates are furnished. Some may even utilize free nitrogen from the air. Other bacteria require organic

nitrogen compounds in the form of amino acids, peptids, peptones, and in a few instances apparently even more complex organic compounds.

Media are sometimes divided into *synthetic* and *nonsynthetic*. A synthetic medium is one in which the exact chemical composition of each of the components is known. A nonsynthetic medium is one in which the exact chemical composition is not fully understood. Some kinds of bacteria can be studied best by the use of the simple synthetic media, but suitable ingredients for such media are not readily available for many other kinds of bacteria.

Synthetic media usually contain as a basis an aqueous solution of certain salts, among them potassium phosphate and sodium chloride. One of the most commonly used is Uschinsky's solution:

Water, distilled	1000 c.c.
Asparagin	4 grams
Ammonium lactate	6 grams
K_2HPO_4	2 grams
NaCl	5 grams

Media of this type have proved very useful in studying the products of fermentation of microörganisms.

Were it possible to secure pure amino acids and peptids in commercial quantities, suitable synthetic media for many of the pathogenic bacteria could be devised which would be of real value in their study and differentiation.

The basis for most laboratory media is nutrient broth or bouillon. It contains either meat infusion or meat extract, peptone, usually salt, and water. *Meat infusion* is prepared by soaking 500 grams of lean minced beef in water in the ice chest for twenty-four hours, filtering the liquid through cheesecloth by means of a meat press, and making up the filtrate to one liter. *Meat extract broth* is prepared by using (in place of the meat infusion) about three grams of commercial beef extract to a liter of water.

Many makes of peptone are upon the market. Peptone

is prepared by the action of the enzyme pepsin of the gastric juice upon proteins. Peptones contain mixtures in varying proportions of albumoses, peptids, and amino acids. Those in which digestion is most complete, that is, those which have the highest percentage of amino acid are frequently most useful in stimulating bacterial growth. However, they cannot be used for certain purposes. In the manufacture of diphtheria toxin, for example, apparently the more complex peptones and albumoses are required. This variation in composition of peptones from different manufacturers and from different lots should be borne in mind in making comparisons in the growth of bacteria grown on media containing these peptones.

Sugars and *glycerin* are frequently added to broth or bouillon in studying the fermentative activities of many species of bacteria, that is, in the study both of acid and of gas production.

Dunham's solution or peptone water is a solution of peptone in water without the addition of meat infusion or beef extract. When the peptone used is of the right composition, that is, when it contains the amino acid tryptophane it may be used for testing the ability of microörganisms to produce the compound indol.

Milk serves as a satisfactory medium for the growth of many species of bacteria. Usually the fresh separated milk is used; whole milk may be kept in an ice chest for twenty-four hours and the milk pipetted from below the cream layer.

Sterile defibrinated *blood* or *blood serum* alone, mixed with sterile broth, are frequently used in media, particularly when it is desired to grow certain of the pathogenic or disease-producing bacteria. Inasmuch as it is not practicable to sterilize serum without coagulation after removal from the body, precautions are taken to insure sterility and

to prevent contamination at the time the blood is drawn. It is then allowed to stand until it clots when the clear serum is pipetted off or it is shaken with glass beads or whipped with a glass rod until defibrinated.

Beer-wort—is probably the most satisfactory medium for cultivation of molds and yeasts. The unhopped wort may be secured from breweries or it may be prepared by soaking malt in warm water. The starch of the malt is rapidly changed into the sugar maltose and certain of the protein constituents likewise pass into solution.

Solid media—Solid media may be divided into three types, media which are permanently firm or solid, media which are at first liquid and later solidified and in which the condition is irreversible, and media which may be liquid or solid at will, that is, solid media which are liquefiable.

To the first group of natural solid media belong materials such as potatoes and other vegetables and the meats. Potatoes when sliced and sterilized prove to be a suitable medium for the growth of many kinds of bacteria.

Blood sera, or mixtures of blood serum with broth, may be solidified by exposure to heat, and constitute satisfactory media for the growth of many of the pathogenic bacteria. *Silicic acid* in pure colloidal solutions may be caused to form a gel by the addition of certain salts. This constitutes, when mixed with these salts, a satisfactory solid medium for the growth of certain of the soil bacteria which develop best in the absence of organic compounds.

The *liquefiable solid media* are prepared usually by the addition either of gelatin or of agar-agar to a liquid medium.

Gelatin is manufactured by boiling bones, joints, tendons, and similar animal tissues, concentrating the extract, and cooling and drying. When dissolved in water it has the property of solidifying or gelatinizing when cool, and of being liquid when heated. Chemically gelatin is closely

related to proteins. Usually from ten to fifteen per cent of gelatin is required in order to cause a medium to solidify when cool. It is not adapted to the solidification of media used for the growth of organisms requiring temperatures higher than average room temperature. Bacteria, for example, which grow only at the temperature of the body cannot well be tested on gelatin as this medium would be usually liquid at this temperature.

Agar-agar is a complex carbohydrate prepared from certain kinds of seaweed. Its solutions resemble those of gelatin in their power of gelatinizing when cool. From one to three per cent is ordinarily used in preparing solid media. It may be noted that the melting temperature and the temperature at which the agar medium solidifies are not identical. A tube of bouillon agar, for example, must be brought nearly to the boiling point before the agar liquefies, but may be cooled to a temperature of forty-two to forty-five degrees before it begins to solidify. The agar-agar is not attacked by microörganisms generally, two or three species only are known which can dissolve or digest it. Gelatin, on the other hand, is readily attacked by many species of bacteria, and one of the differential characters noted is the ability of certain forms to digest or liquefy the gelatin.

By the addition of agar or gelatin practically any of the ordinary liquid media may be solidified.

Many other types of media and variations in the media thus far mentioned will be noted later in the discussion of the growth requirements of the specific kinds of bacteria.

Adjustment of the Reaction of the Medium

The reaction of a medium, that is, its relative acidity or alkalinity, may be designated in one of two ways: first, by the amount of normal acid or normal alkali required to bring one hundred cubic centimeters of the medium to the

neutral point of some particular indicator, or second, by the designation of the true hydrogen ion concentration or, conversely, of the true hydroxyl ion concentration of the medium. While the first method is still the more commonly used, the second method is in most instances preferable.

Bacteria, yeasts, and molds are usually quite sensitive to the presence of an excess of acid or alkali. Some grow best in a medium which is strictly neutral, others prefer one which is somewhat on the acid side of neutrality, still others on the alkaline side of neutrality. Careful adjustment of reactions is, therefore, necessary in many cases. Some organisms, for example, will not grow unless the medium has almost exactly the same reaction as does the blood or the tissues of the body in which they are accustomed to grow.

The chemist tells us that acidity is determined by the presence of free hydrogen ions, alkalinity is determined by the presence of free hydroxyl ions, and that a solution is truly neutral when equal numbers of hydrogen and hydroxyl ions are present in a given volume. *Pure* distilled water is neutral. This does not mean that it has no free hydrogen ions and no free hydroxyl ions, but that when molecules of water dissociate they break up into equal numbers of each ion. The chemist has discovered, furthermore, that pure water (and therefore any neutral solution of materials in water) contains approximately *one ten-millionth of a gram of hydrogen ions to the liter.* This may also be written 10^{-7} grams hydrogen ions per liter, or still more conveniently expressed in terms of normality of hydrogen ions. A normal solution of hydrogen ions is one which contains one gram of hydrogen ions per liter. A neutral solution, therefore, is one which has a concentration of hydrogen ions of 10^{-7} normal. It was noted above that in a neutral solution there is the same number of hydroxyl ions as hydrogen ions. The hydroxyl ion concentration must, therefore, be also 10^{-7} normal.

The physical chemist has also discovered that the product of the normality of hydrogen ions by the normality of hydroxyl ions is a constant number. What this number is may be determined by multiplying 10^{-7} (normality of hydrogen ions in a neutral solution) by 10^{-7} (normality of hydroxyl ions in a neutral solution) giving 10^{-14}. In other words, the product of the normality of the hydrogen ion concentration of a solution by the normality of the hydroxyl ion concentration must always be approximately 10^{-14}. If we know either the hydroxyl or hydrogen ion concentration we can at once determine the concentration of the other. For example, if we are dealing with a solution having an hydrogen ion concentration of 10^{-5} normal its hydroxyl ion concentration must be 10^{-9} normal. It is thus possible to arrange a scale to designate the acidity of any solution in terms of its hydrogen ion concentration as follows:

10^{0} 10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} 10^{-9} 10^{-10} 10^{-11} 10^{-12} 10^{-13} 10^{-14}.

On this scale it will be noted that the larger the numerical value of the exponent, the smaller the hydrogen ion concentration. It will be recalled that 10^{-7} represents neutrality. Numbers to the right of 10^{-7} represent increasing values of alkalinity or decreasing hydrogen ion concentration. Numbers toward the left represent increasing acidity. Inasmuch as this method of statement is somewhat cumbersome, it has been suggested by Sörenson that the exponents be used to indicate the scale, using positive signs instead of negative. This gives the scale:

0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14.

Each of these numbers is termed the pH of a solution. A solution having pH of 0, for example, would have a normality of hydrogen ion concentration of 10^{0} normal or 1. One having a pH value of 7 would have a hydrogen ion concentration of 10^{-7} normal, that is, it would be neutral. It is evident, therefore, that the smaller the number in

this scale, the higher the hydrogen ion concentration, that is, the greater the acidity; and the larger the number, the greater the alkalinity.

Indicators are chemical substances which are of one color in a certain range of pH values or hydrogen ion concentrations, and of another color in other ranges. The indicator most commonly used is litmus, which has a lilac color at true neutrality, that is, at a pH value of 7. At a pH value of 8, that is, in a more alkaline solution, it is blue. At a pH value of 5, that is, in a stronger concentration of hydrogen ions, it is red. Other indicators change color at other pH values. For example, phenolphthalein is colorless in all hydrogen ion concentrations having a pH value less than 8.2; in more alkaline solutions, it is red. Many other indicators are known which change color at other points in the hydrogen ion scale. This change of color is not instantaneous. In adding alkali, for example, to an acid solution containing litmus, there is not an instantaneous transformation of red into blue. By the use of standards whose hydrogen ion concentration is known it is possible to determine approximately the hydrogen ion concentration of any material which it is desired to test, by the use of the intensity of color of appropriate indicators.

Most bacteria grow in a hydrogen ion concentration of 10^{-7} to 10^{-8}, that is, in solutions having a pH value between 7 and 8. The pH value of blood is usually about 7.35 This indicates, therefore, the hydrogen ion concentration most useful in cultivating many species of pathogenic bacteria. Inasmuch as most media are on the acid side of true neutrality it is necessary to add alkali, usually potassium or sodium hydrate, until test shows that the hydrogen ion concentration has become satisfactory.

The hydrogen ion concentration of medium or solution depends not only upon the actual concentration and kind of the acid but upon the concentration of substances which

are termed *buffers*. A buffer is any substance in a solution which tends to prevent rapid changes in hydrogen ion concentration upon the additions of alkalies or acids. Certain salts, particularly the phosphates, and many organic substances, particularly the amino acids and peptones, act in this manner. For example, the addition of a small amount of an acid to distilled water will give a marked change in the hydrogen ion concentration, but the same amount of acid added to solutions containing considerable amounts of buffers may result in very slight differences in hydrogen ion concentration. It is evident that since microörganisms are affected far more by differences in hydrogen ion concentration than they are by the total amount of acid present, heavily buffered media are preferred for their growth.

It is noted above that media are sometimes standardized by determining the amount of acid or alkali required to bring a hundred cubic centimeters to the neutral point of some indicator. The one usually chosen in bacteriology is phenolphthalein. A solution is said to be —1, for example, when it will require one cubic centimeter of a normal solution of acid to bring it to the neutral point of phenolphthalein. A heavily buffered medium, such as ordinary peptone broth, having a reaction of +1 or +1.5, usually has a pH value between 7 and 8. Direct determination of hydrogen ion concentration is preferable to titration in adjusting the reaction of the medium.[1]

When a medium has been finally adjusted in its reaction, that is, when the right amount of alkali or acid has been added to give the desired hydrogen ion concentration, it is frequently necessary to boil or heat the medium to precipitate any materials not soluble in boiling water at the new hydrogen ion concentration. This is followed by filtration. If this step is omitted the medium will ordinarily be cloudy or full of sediment.

[1] For methods of adjusting reactions see laboratory manual.

After being placed in suitable containers the medium is then ready for sterilization. The method used will depend upon the character of the medium. Most media are sterilized by heating in the autoclave at one atmosphere pressure for from ten to fifteen minutes. Care must be used in the sterilization of sugar media, however, because at certain hydrogen ion concentrations they are rapidly decomposed, the disaccharides in particular being hydrolyzed to monosaccharides. Some media are sterilized by exposure either for one long period or for short periods on successive days, to streaming steam. Still other media, particularly those containing blood serum, are solidified by heating at a temperature of from 75° to 80° C., and sterilized by exposure to these temperatures on several successive days. In some cases it is necessary to sterilize the ingredients of the medium separately and mix them in the right proportions after sterilization.

Pure Culture Methods

It has previously been noted that it is exceptional in nature to find a single kind of microörganism growing by itself, that is, free from all other kinds. This sometimes occurs with disease-producing bacteria in the blood or tissues, and under some exceptional conditions of environment it may be possible for only one kind of germ to grow. It is evident, therefore, that if bacteria are to be studied they usually must first be separated from each other and grown in pure cultures. A *mixed* or *impure* culture is one in which several kinds of organisms are growing together.

It is possible sometimes to secure pure cultures by *direct transfer*. For example, it is usually possible to get a pure culture of a mold such as Mucor by touching a sterile needle to the sporangia. A few spores will adhere and these may be scattered over the surface of the culture medium upon which the organism is to be grown. If the medium is suitable some at least of the young mold plants developing will

be free from contamination, that is, they will be pure. It is much more difficult to secure pure cultures of bacteria in this mechanical fashion. It may be accomplished, however, when the necessity arises, by the use of *Barber's capillary pipette.* This is an instrument consisting of an extremely slender capillary glass tube so arranged with a thumb screw that water can be forced in and out. The bacteria to be separated are placed in a drop of water and examined under the microscope. By careful manipulation the germ desired may be drawn into the capillary pipette and blown out again into a drop of water free from bacteria. Then it may be examined to be sure that there are no other bacteria present, when it may be transferred to the medium upon which it is to be cultivated. This method is cumbersome and the technic is difficult. It has proved valuable for certain special studies but is not in common use.

Perhaps the most common method of securing pure cultures of bacteria is by *plating.* The mixture of bacteria to be separated is placed in liquid nutrient agar or nutrient gelatin, that is, in one of the liquefiable culture media. When shaken thoroughly so that the bacteria are well distributed, this is poured into a sterile glass Petri dish and covered with a sterile glass cover. The separated bacteria are soon fixed in position by the hardening of the culture medium. They can no longer move about, but their growth is not interfered with. In consequence they develop rapidly and in the course of a few hours to a few days produce a mass of bacteria large enough so that it can be easily seen with the naked eye. The mass of organisms is termed a *colony.* If the number of bacteria introduced into the original medium is not too large and they are properly mixed through the medium the colonies will be well separated from each other. Theoretically, at least, each organism gives rise to a separate colony, prac-

tically, bacteria sometimes stick together, and a colony will occasionally consist of more than one kind of organism. In the majority of instances, however, each colony consists of a pure culture.

A method closely related to the one just described for purification of bacterial cultures is that of *streaking.* A solid medium such as agar is prepared and the bacteria to be separated smeared back and forth over its surface in an effort to separate the cells from each other. When successfully done the individual species may be separated in colonies and pure cultures secured.

It is sometimes possible to use as a medium for plating or for streaking some material which will not allow of the growth of any organisms but the particular one desired. Media of this type are termed *differential media.* For example, ox bile is sometimes used to eliminate bacteria which are not of intestinal origin. Some kinds of pathogenic organisms may be secured in pure culture by the device of animal inoculation. If, for example, it is desired to secure a pure culture of the *Mycobacterium tuberculosis,* the organism causing tuberculosis in cattle, from a sample of milk, this may be accomplished most readily by inoculating some of the milk containing the organism into a suitable animal, particularly, perhaps best, into a guinea pig. Within a few weeks the animal may be killed and the organism will be found growing in pure culture in the lymph glands.

When once colonies consisting of one kind of organism only have been secured, these may be transferred to other tubes of media by means of the platinum loop or the platinum wire. By successive transfers, it is possible to keep most kinds of bacteria growing indefinitely in the laboratory.

Before any important study of an organism is undertaken it is customary to plate or in some other way deter-

mine whether or not it is still pure. Inasmuch as microörganisms are common in the air there is always a chance that some of the air forms may gain entrance when tubes of media are opened. Many serious errors have been made by students and investigators because they were dealing not with pure cultures but with mixtures of various species.

Study of Growth Characters

It is relatively difficult to tell many kinds of microörganisms apart, consequently every observable point of difference should be noted. Wherever possible bacteria are differentiated by observation of size, shape, motility, spore production, and other morphologic characters. However, these frequently fail, and species must be differentiated from each other by their appearance when grown upon culture media, and by differences in their behavior, that is, in their physiologic characteristics.

For the purpose of arranging in logical order the most important of the morphologic, cultural, and physiologic characteristics of an organism, a committee of the Society of American Bacteriologists has evolved a descriptive chart. A copy of this chart is included on pages 88 to 90. First on the chart is the name of the the organism, the correct genus and species to be given together with the authority, when this can be determined. Next, it will be noted, a statement is inserted concerning invigoration of culture. Bacteria that have been grown for some time on unsuitable laboratory media sometimes need rejuvenation, that is, cultivation upon some unusually suitable medium for a period in order that they may regain their vigor.

The cultures to be studied are usually slant or streak cultures on solid media, stab cultures and plate cultures, in gelatin or agar, or cultures in a liquid medium such as broth or milk.

DESCRIPTIVE CHART

FOR USE IN BACTERIOLOGICAL INSTRUCTION

Recommended by the Committee on the Chart for Identification of Bacterial Species at the 1917 meeting of the

SOCIETY OF AMERICAN BACTERIOLOGISTS

H. J. Conn
H. A. Harding
I. J. Kligler
W. D. Frost
M. J. Prucha
K. N. Ayers
} Committee

Name of organism ..... Culture No. .....

Source ..... Studied by .....

Date of isolation.....

Invigoration of Culture

Date.....

Series No.....

Medium used.....

Temperature.....°C. Number of transfers .....

Length of each incubation..... days.

MORPHOLOGY

Note—Underscore required terms.

Sketches

Vegetative Cells, Medium used..... temp..... age ..... days.
Form, *spheres, short rods, long rods, filaments, commas, short spirals, long spirals, curved.*
Arrangement, *single, pairs, chains, fours, cubical packets.*
Limits of Size.....Size of Majority.....
Ends, *rounded, truncate, concave*
Capsules, present on.....
How stained .....

Sporangia, *present, absent.* Medium used..... temp..... age ..... days.
Form *elliptical, short rods, spindled, clavate, drumsticks*
Limits of Size.....Size of Majority.....
Endospores, *present, absent.*
Location of Endospores, *central, polar.*
Form, *spherical, elliptical, elongated.*
Limits of Size.....
Size of Majority.....
Wall, *thick, thin*
Sporangium wall, *adherent, not adherent.*

Motility
In broth.....Orange .....
Flagella, No. .....Attachment, *polar, bipolar, peritrichate,* How stained .....

Irregular Forms,
Present on.....in... days at .....°C.
Form *spindled, sumeate, filamentous, branched,* or .....

Staining Reactions.
1-10 watery fuchsin, gentian violet, carbol fuchsin, Loeffler's alkaline methylene blue.
Special Stains.
Gram.....Acid fast.....
Neisser .....

BRIEF CHARACTERIZATION

Group No. .....

GROUP NUMBER

As each of the determinations listed below is made, check the proper figure. When complete place the entire group number in the space above.

100.	Endospores produced
200.	Endospores not produced
10.	Aerobe (Strict)
20.	Facultative anaerobic
30.	Anaerobe (Strict)
1.	Gelatin liquefied
2.	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
0.4	No growth with dextrose
.01	Acid and gas from lactose
.02	Acid without gas from lactose
.03	No acid from lactose
.04	No growth with lactose
.001	Acid and gas from saccharose
.002	Acid without gas from saccharose
.003	No acid from saccharose
.004	No growth with saccharose
.0001	Nitrates reduced with evolution of gas
.0002	Nitrates reduced without gas
.0003	Nitrates not reduced
.00001	Fluorescent
.00002	Violet chromogens
.00003	Blue "
.00004	Green "
.00005	Yellow "
.00006	Orange "
.00007	Red "
.00008	Brown "
.00009	Pink "
.00000	Non-chromogenic
.000001	Diastatic action on starch, strong
.000002	Diastatic action on starch, feeble
.000003	Diastatic action on starch, absent
.0000001	Acid and gas from glycerin
.0000002	Acid without gas from glycerin
.0000003	No acid from glycerin
.0000004	No growth with glycerin

The genus according to the system of Migula is given its proper symbol which precedes the number thus:

Bacillus coli (Esch.) Mig. becomes B. 223.111202

Vegetative Cells		Diameter over 1μ
		Diameter 0.5–1μ
		Diameter under 0.5μ
		Length over two diams
		Chains (4 or more cells)
		Filaments
		Capsules
		Motile
		Gram's Stain
Endospores		Central
		Polar
		Diameter > Diam of rod
		Round
		Oval to cylindrical
		Absent
Cultural Features	Agar Streak	Abundant
		Absent
		Shining
		Wrinkled
		Chondrogenic
	Agar Colonies	Punctiform
		Round (over 1 mm.)
		Rhizoid
		Filamentous
	Gel. Colonies	Curled
		Punctiform
		Round (over 1 mm.)
		Irregular
		Filamentous
Physiology	Milk	Acid curd
		Rennet curd
		Casein peptonized
		Acid without curdling
		Unchanged
	Fluorescent	
	Luminous	
	Animal pathogen	
	Plant pathogen	
	Saprophyte	

FERMENTATION

Temperature.....°C.

Fermentation-tubes containing .....and:	DEXTROSE	LACTOSE	SACCHAROSE	GLYCERIN				
Growth in closed arm								
Gas percentage								
$\frac{H}{CO^2}$								
Acid in.....days								
Acid in.....days								
Acid in .....days								

MILK

Temperature..... °C.

REACTION	COAGULATION	PEPTONIZATION
1 day.....	1 day.....	1 day.....
2 days	2 days	2 days .....
4 days	4 days	4 days .....
7 days	7 days	7 days .....
10 days	15 days	10 days .....

LITMUS MILK

Temperature .....°C.

REACTION	COAGULATION	PEPTONIZATION	REDUCTION
1 day	1 day.....	1 day.....	1 day.....
2 days	2 days	2 days	2 days .....
4 days	4 days	4 days	4 days .....
7 days	7 days	7 days	7 days .....
10 days	10 days	10 days	10 days .....

NITRATE REDUCTION

Medium:..... Temperature.....°C.

Nitrite: 1 day.....; 2 days.....; 4 days.....; 7 days.....; 10 days.....

Gas: 1 day.....; 2 days.....; 4 days.....; 7 days.....; 10 days.....

CHROMOGENESIS

Nutrient broth	
Nutrient gelatin	
Nutrient agar	
Potato	

DIASTATIC ACTION

Breadth of clear zone on starch agar plates
in.....days:
in.....days:
in.....days:

TEMPERATURE RELATIONS

Optimum temperature for growth .....°C.

Maximum temperature for growth .....°C.

Minimum temperature for growth .....°C.

CULTURAL CHARACTERISTICS

	……hours	Changes, ……days	Changes, ……days	SKETCHES
Agar Streak Incubation Temperature ……°C	Growth, *scanty, moderate, abundant, none.* Form of growth, *filiform, echinulate, beaded, spreading, arborescent, rhizoid.* Elevation of growth, *flat, effuse, raised, convex.* Lustre, *glistening, dull.* Topography, *smooth, contoured, rugose.* Optical Characters, *opaque, translucent, opalescent, iridescent.* Chromogenesis……Photogenic. Fluorescent. Odor, *absent, decided, resembling* …… Consistency, *butyrous, viscid, membranous, brittle.* Medium, *grayed, browned, reddened, bleed, greased.*			
Gelatin Stab Temperature ……°C	Growth, *uniform, best at top, best at bottom.* Line of puncture, *filiform, beaded, papillate, villous, arborescent.* Liquefaction, *none, crateriform, napiform, infundibuliform, saccate, stratiform;* begins in……d, complete in……d. Depth of liquefaction in tube of 10 mm. diameter, evenly inoculated at 20° C. for 30 days……mm. Medium, *fluorescent, browned.*			
…… Medium (solid) Temperature ……°C				

	……hours	Changes, ……days	Changes, ……days		……hours	Changes, ……days	Changes, ……days
Nutrient Broth Temperature ……°C	Surface growth, *ring, pellicle, flocculent, membranous, none.* Clouding, *slight, moderate, strong transient, persistent, none, fluid turbid.* Odor, *absent, decided, resembling* …… Sediment, *compact, flocculent, granular, flaky, viscid on agitation, abundant, scant, none.*			…… Medium (liquid) Temperature ……°C			

		SKETCHES			
		Surface Colony		Deep Colony	
— Agar Colonies Temperature ……°C	Growth, *slow, rapid.* Form, *punctiform, circular, irregular, mycloid, filamentous, rhizoid.* Surface, *smooth, rough, concentrically ringed, radiate.* Elevation, *flat, effuse, raised, convex, pulvinate, umbonate.* Edge, *entire, undulate, lobate, erose, filamentous, curled.* Internal structure, *amorphous, finely-, coarsely-granular, filamentous, curled, concentric.*	….days	….days	….days	….days
Gelatin Colonies Temperature ……°C	Growth, *slow, rapid.* Form, *punctiform, circular, irregular, mycloid, filamentous.* Elevation, *flat, raised, convex, pulvinate, crateriform (liquefying).* Edge, *entire, undulate, lobate, erose, filamentous, floccose, curled.* Liquefaction, *cup, saucer, spreading.* Internal structure, *amorphous, finely-, coarsely-granular, filamentous, curled, concentric.*	….days	….days	….days	….days

On the second page of the descriptive chart will be found the characteristics to be observed in the various cultures, and some of the more common terms used to designate these.

CHAPTER VII

METHODS OF STUDYING PHYSIOLOGIC CHARACTERS

Many species of microörganisms are differentiated from each other on the basis of differences in their physiologic characters. For example, certain closely related disease-producing bacteria are differentiated because of their ability to produce acid or gas from certain sugars. A study of these characters teaches something of the parts which these microörganisms play in nature in bringing about chemical changes. Only a few of the most important of these physiologic reactions and characters are here discussed, many others will be noted in subsequent chapters. Those here given are the ones most frequently used in the differentiation of species.

A study of physiologic characters of bacteria is largely a study of specific chemical changes brought about by these microörganisms. The characters most frequently used in the laboratory are changes in hydrogen ion concentration, determination of acid production, alcohol production, oxygen relationship, gas production, reduction changes, production of indol, digestion of starch and other insoluble carbohydrates, digestion of proteins, nitrogen fixation, oxidation of ammonia to nitrites, and oxidation of nitrites to nitrates.

Determination of Changes in Hydrogen Ion Concentration.—Many bacteria when grown in nutrient solutions, particularly those containing carbohydrates, bring about marked changes in hydrogen ion concentration. The amount of such change accomplished by a given organism depends upon several factors, the most important being the following: first, the amount of acid produced, second

the kind of acid produced, third, the amount of buffer present in the nutrient medium, fourth, the amount of alkali (that is, the concentration of hydroxyl ions) produced at the same time. Alkali may be developed either by the production of ammonia or by the transformation of the salt of a strong acid to the salt of a relatively weak or little dissociated acid. For example, certain bacteria may transform sodium citrate into sodium carbonate, the latter being decidedly alkaline in its reaction.

Two methods are in common use for detecting changes in hydrogen ion concentration. The first is by a determination of the electric conductivity. The second is a colorimetric method. For usual laboratory routine the colorimetric method is the simpler and is the only one which will be discussed. In this method it is customary to add a suitable indicator either to the solution to be tested or to a portion of this solution diluted somewhat with distilled water. The color secured is then compared with the color produced by similar addition of indicator to standard solutions whose hydrogen ion concentration is known. The indicators most used in the bacteriology laboratory for this purpose are those developed by Clark and Lubs. In Table I the name of each of these indicators is given followed by the color in its acid range, next the color in its alkaline range, and finally the range of pH values through which it changes color.

It is apparent that an approximate idea can be secured of the change in hydrogen ion concentration by using different indicators and determining which gives an acid color and which an alkaline color. For example, if it is found that phenol red gives a yellow reaction the medium is acid and must be below the pH value of 6.8. If methyl red, on the other hand, gives an alkaline color it must have a pH value above 6. The exact pH value can be determined then by the use of brom thymol blue, comparing the

COLOR CHANGES OF CLARK AND LUBS INDICATORS

Indicators	Full acid color	Full alkaline color	Sensitive range. The indicator changes from the acid color to the alkaline color between the following PH values
Thymol blue (Acid range)	Red	Yellow	1.2 – 3.8
Brom phenol blue	Yellow	Blue	3.0 – 4.6
Methyl red	Red	Yellow	4.4 – 6.0
Brom cresol purple	Yellow	Purple	5.2 – 6.8
Brom thymol blue	Yellow	Blue	6.0 – 7.6
Phenol red	Yellow	Red	6.8 – 8.4
Cresol red	Yellow	Red	7.2 – 8.8
Thymol blue (Alkaline range)	Yellow	Blue	8.0 – 9.6
Phenolphthalein	Colorless	Red	8.0 – 9.8
Cresolphthalein	Colorless	Red	8.2 – 9.8

intensity of the color change from yellow to blue with standard solutions whose pH values are known.

In figure 48 is given diagrammatically a scale of pH values from 1 to 14. This scale is designated to the left. To the right are designated the pH values of certain solutions and of media commonly used in the laboratory. The heavy portions of the lines designating the indicators show those particular ranges in which the color changes are most rapid. It should be remembered that a decrease of 1 in pH value multiplies the hydrogen ion concentration by ten. A pH of 0 means normal concentration of hydrogen ions, and a pH of 14 a normal solution of hydroxyl ions.

FIG. 48.—THE EFFECTIVE RANGE OF INDICATORS MOST COMMONLY USED IN THE BACTERIOLOGICAL LABORATORY.

For discussion of the methods of preparing standard solutions and colors for accurate determinations for hydrogen ion concentrations a suitable laboratory manual should be consulted.

Determination of Acid Production.—What has been stated above concerning determination of hydrogen ion concentration will indicate the method of determining whether or not acid has actually been produced by an organism. It should be noted, however, that the determination of hydrogen ion concentration is not a determination of the total amount of acid produced. This can be determined only by a comparative titration. It is customary to titrate to a definite tint a sample of the sterile medium retained as a check, using phenolphthalein as an indicator. For this purpose it is usual to place 5 cc. of the sterile material mixed with 45 cc. of distilled water in a porcelain evaporating dish, add a drop or two of alcoholic solution of phenolphthalein and add twentieth normal (N/20) alkali until a definite pink color has been established. This is kept as a standard, and the amount of alkali required noted. A similar titration is made using the medium in which the organism to be studied has been grown. This is treated in exactly the same fashion and the twentieth normal alkali added until the same tint has been secured as with the check. The differences between the amounts of alkali required for bringing to the same tint of red will vary directly with the amount of acid produced. This may be calculated in grams per liter if the kind of acid formed is known.

This method of acid determination is not absolutely accurate inasmuch as there may be alkalies developed simultaneously with formation of acid. There is no simple method of correcting for this possible error.

In some cases it is desirable to determine the kind of acid which has been produced. Simple chemical tests for

the various organic acids have not in most instances been developed. Something of their nature, however, may be determined by separating them into volatile and nonvolatile. If a solution containing an acid is acidified with sulphuric acid and the materials distilled, certain acids, particularly acetic, propionic and butyric will pass over, while other acids, particularly lactic, will not. This makes it possible by titration of the distillate to determine the relative proportion of volatile and nonvolatile acid.

Determination of Alcohol Production.—Yeasts produce considerable quantities of ethyl alcohol, and smaller amounts are formed as a result of the growth of a few bacteria and molds. Occasionally other alcohols may be developed such as amyl, butyl and propyl. With yeasts alcoholic fermentation is accompanied by the production of carbon dioxide. The presence of ethyl alcohol may be detected by placing a few cubic centimeters of the material to be tested in a suitable container such as a test tube, and adding a small crystal of iodine and several cubic centimeters of a strong solution of sodium hydrate. If alcohol is present heating this material over a Bunsen flame will give a distinct odor of iodoform. The amount of alcohol developed may be determined by distillation and test of the specific gravity of the distillate.

Determination of Aldehyde.—Certain microörganisms, particularly when growing in carbohydrate solutions, produce aldehyde in sufficient quantities to give a distinctive reaction. The detection of the presence of aldehyde is best accomplished by the use of a fuchsin indicator. If a solution of basic fuchsin is decolorized by the addition of sodium sulphite (or better suphurous acid) until the color has just disappeared or until the material is of a very light pink color, and added to a solution in which aldehyde has developed, the color of the fuchsin is restored. This is the principle made use of in the so-called Endo medium.

Certain bacteria when growing upon this medium form red colonies because the fuchsin turns red as a result of the development of aldehyde and acid. Other species of bacteria grown upon this medium do not change the color at all.

Production of Acetyl Methyl Carbinol.—This compound is produced by certain bacteria growing in the presence of carbohydrates. It is recognized by the addition of strong alkali such as sodium hydrate or potassium hydrate. When allowed to stand for a few hours an eosin pink or red color will develop, particularly near the surface, providing there is some peptone present. This is frequently called the Voges-Proskauer reaction, after the men who first noted it.

Determination of Oxygen Relationships.—Bacteria are frequently divided into two groups, those which require free oxygen of the air for their development and those which will grow without. The first are termed *aërobes*, the second *anaërobes*. Those bacteria which can grow either in the presence or the absence of free oxygen are termed *facultative* anaërobes, and those which will not grow in the presence of free oxygen are termed *obligate* anaërobes.

It is frequently necessary in the laboratory to determine accurately the oxygen preferences of a microörganism. The bacteria which will grow only upon the surface of a medium, but never grow in the closed arm of a fermentation tube under any conditions, are the obligate aërobes. For the most part these are organisms which are actual oxidizers, changing carbonaceous materials to carbon dioxide and water, for example. The facultative anaërobes are those which will grow in contact with air, but, at least under certain conditions, will grow in the absence of free atmospheric oxygen. Most of the facultative organisms are obligate aërobes on certain media and facultative on others. Many bacteria, for example, which usually require

atmospheric oxygen can grow in its absence providing nitrates are present to furnish an available supply of oxygen though not in the free form. Other bacteria will grow in the absence of oxygen providing they have suitable carbohydrates. For example, the organism known as *Bacterium coli* will grow only in the open arm of a fermentation tube if sugar is absent, but in the presence of a suitable sugar such as dextrose it grows both in the open and in the closed arm.

The obligate anaërobes are those which will grow only in the absence of free oxygen or are definitely injured by its presence. It is evident that special cultural conditions are necessary for their study.

A few bacteria have been termed *microaërophiles* because they tend to grow in a definite concentration of oxygen. When mixed with melted agar in a test tube and the agar allowed to solidify, they will grow in a definite zone some distance below the surface of the medium.

Determination of Gas Production.—Bacteria which require free oxygen for their development frequently produce considerable quantities of carbon dioxide. Inasmuch as they are growing in contact with air, however, this gas diffuses into the air and does not collect as gas bubbles in the medium. When it is desired to study gas production by such bacteria it is necessary to grow them in a closed vessel to which air enters through a small opening and leaves through a tube which then passes through a suitable absorbing agent, for example, strong sodium hydrate, which will collect the carbon dioxide. The amount of gas may be calculated by the increased weight of the sodium hydrate.

Most of the bacteria producing considerable amounts of gas in laboratory media are anaërobes or facultative anaërobes. The gases developed depend somewhat on the composition of the medium, and also upon the character of the

organism. Carbon dioxide, hydrogen, nitrogen, and methane are the most common gases formed.

As a test for the ability of an organism to produce gas we may inoculate a liquid culture of agar or gelatin containing a suitable carbohydrate. The development of gas will cause the formation of numerous bubbles. A qualitative determination of the kinds of gas developed may be made by use of a fermentation tube containing a suitable liquid carbohydrate medium. The closed arm is entirely filled with the medium. Organisms grown in the closed arm will produce gas which will accumulate in the upper part of this closed arm. The amount produced may be readily estimated by noting the proportionate part of the length of the tube displaced by gas. A qualitative test may be made by filling the open arm with normal sodium hydrate, closing the open arm, shaking the gas into the open arm, returning the gas to the closed arm, and removing the cork. The liquid will rise to replace any carbon dioxide which has been produced. Yeasts and some bacteria produce carbon dioxide only. With these the liquid will rise practically to the top of the tube after the absorption of the gas. Many bacteria produce both hydrogen and carbon dioxide. The hydrogen may be measured after the absorption of the carbon dioxide by the alkali. When drawn into the open arm, mixed with air, and brought near to a flame, it will explode. A few microörganisms growing in the presence of nitrates produce considerable amounts of free nitrogen gas.

Methane is produced by a few bacteria, but not by organisms most commonly grown in the laboratory.

Accurate studies of the comparative amounts of carbon dioxide and hydrogen produced by bacteria have proved useful in some cases in differentiating them. Such studies can be made only by the use of an apparatus filled with medium and containing no gas, sealed to prevent the escape

of gas until the material is subjected to chemical analysis. As the result of such a chemical test, the ratio of carbon dioxide to hydrogen may be determined.

Determination of Reduction Changes.—It has already been noted that certain facultative and other bacteria growing in the absence of free oxygen, may take oxygen from certain compounds, reducing nitrates to nitrites, sulphates to sulphides, and decolorizing certain pigments, such as litmus or methylene blue. The facts are sometimes of advantage in the differentation of bacteria.

Reduction of Nitrates to Nitrites—The transformation of nitrates into nitrites may take place in a number of different ways as the result of growth of microörganisms. The test is, therefore, not particularly reliable and is not emphasized by bacteriologists as it was formerly. A peptone broth containing usually two-tenths per cent of potassium nitrate may be inoculated with the organism to be studied. After standing for four days the nitrite test may be performed by adding two cubic centimeters of each of the following solutions:

(a) 5N Acetic acid,.....1000 cc.
Sulphanilic acid.....8 grams
(b) 5N Acetic acid.....1000 cc.
Alpha amido napthalene.....5 grams

If nitrite is present a reddish or rose color will develop. It is always necessary to test an uninoculated tube at the same time as a check to be sure that no nitrites were initially present in the medium used.

Reduction of Sulphates to Sulphides—Some organisms reduce sulphates to sulphides under anaërobic conditions. Media may be prepared containing either lead acetate or iron chloride. The production of sulphides from sulphates will be evident by the blackening of the medium. It should be recognized, however, that sulphides may also originate during the decomposition of organic sulphur compounds.

In studying the reduction of sulphates to sulphides, therefore, a medium should be chosen which does not contain any organic sulphur. Hydrogen sulphide may be recognized by heating the medium and exposing lead acetate paper to the vapor. The presence of hydrogen sulphide will be shown by the darkening or blackening of this paper.

Reduction of Pigments—Certain pigments, such as litmus and methylene blue, may be partially or completely decolorized by the growth of organisms under anaërobic conditions. This fact is made use of in litmus milk. Many bacteria will completely reduce the litmus to its leuco base. The ability of microörganisms in the presence of organic matter to reduce methylene blue has been made the basis for a test to determine the amount of putrescible organic matter present in solution. Raw sewage, for example, if shaken up with methylene blue and corked tightly in a bottle, will soon become decolorized. The amount of pollution in the sewage or amount of organic material present in the sewage is a function of the length of time which it will take the methylene to become decolorized under standard conditions. The time which passes before the blue color completely disappears is usually regarded as inversely proportional to the amount of organic matter present which may be decomposed readily.

Determination of Indol.—The compound indol is produced by certain species of bacteria from the amino acid tryptophane. This tryptophane in turn is a product of the hydrolysis of certain proteins. Some organisms are capable of producing indol from proteins, that is, they are capable of hydrolyzing the proteins, breaking them down into their component amino acids, including tryptophane, and then decomposing tryptophane with the development of indol. Most of the indol-producing bacteria, however, are incapable of attacking the proteins and must have tryptophane present before they can produce indol. The

various peptones on the market differ considerably in their content in amino acid and tryptophane. For demonstration of this property a peptone relatively high in tryptophane should be chosen.

The development of indol from tryptophane is markedly influenced by the presence of other substances. Most bacteria, for example, which can produce indol from tryptophane will not bring about this change in the presence of carbohydrates.

Indol has a characteristic, disagreeable, fecal odor. Many different chemical tests have been suggested for its detection. The simplest of these is the nitroso-indol test, which is carried out by adding a cubic centimeter of a 0.1 per cent solution of sodium nitrite together with a few drops of strong acid such as sulphuric to the medium. The nitrite is decomposed by the sulphuric acid, freeing nitrous acid. This in turn combines with indol to form nitroso-indol which has a bright red color. For other laboratory tests for indol, and for methods of detection in very small quantities the student should consult a laboratory manual.

Some bacteria are capable of producing the closely related compounds skatol and phenol from tryptophane.

Digestion of Starch and Other Insoluble Carbohydrates.—Many bacteria when grown upon potato or in agar or other media containing starch hydrolize the starch or ferment it more or less completely. The ability to bring about this change is termed the diastatic power of the microörganism. It may be recognized by using an agar containing enough starch to make it somewhat opaque. The organism when grown upon this medium will produce a clear area immediately about the colony. This may be emphasized by the addition of a solution of iodine, when the undigested or unfermented starch will turn blue, leaving a colorless zone about the colony. Not all bacteria hydrolize the starch with the production of sugars.

Other insoluble carbohydrates may be substituted for starch in culture media. Finely powdered cellulose or hemicellulose, for example, may be used in agar to test the ability of microörganisms to digest these substances.

Digestion of Proteins.—Many microörganisms have the power of liquefying gelatin due to the production of an enzyme called gelatinase. This is best observed in stab cultures in test tubes. A few bacteria possess the power of liquefying coagulated proteins, such as blood serum. Some bacteria are able to digest casein in milk bringing about a peptonization of this material. This latter phenomenon may also be demonstrated by the use of a casein agar, that is, nutrient agar to which casein has been added. This medium is somewhat opaque in appearance. Those organisms which digest the casein will clear the medium for a certain distance around the individual colonies.

Fixation of Atmospheric Nitrogen.—Usually bacteria which grow luxuriantly in a nutrient solution containing no compounds of nitrogen are those which are capable of securing their needed nitrogen from the atmosphere. Quantitative tests for amount of nitrogen fixation can be made only by chemical analyses of the culture medium when the organism is well grown. In soil bacteriology it is frequently customary to use the soil itself as a culture medium, applying a suitable carbohydrate to supply growth energy to the nitrogen fixing bacteria. Comparative analyses of soils which have received a carbohydrate with others kept under the same conditions but without this amendment will indicate the amount of nitrogen fixed.

Oxidation of Ammonia and of Nitrites.—Certain soil bacteria are capable of oxidizing ammonia to nitrites. When they are grown in a suitable solution containing ammonium sulphate without organic nitrogen, the development of the nitrite may be observed by using the test above indicated for the detection of nitrites.

Other soil bacteria are capable of oxidizing nitrites to nitrates. Observation of this change is frequently made in soil cultures. Chemical analyses may be made at intervals to show the development of this compound.

CHAPTER VIII

MICROSCOPIC METHODS

THE fact that bacteria, yeasts, and molds are so minute necessitates the constant use of the microscope in the investigation of the size, shape, and arrangement of cells of bacteria, the form of yeast cells, and the method of spore production and general morphology of the molds. For

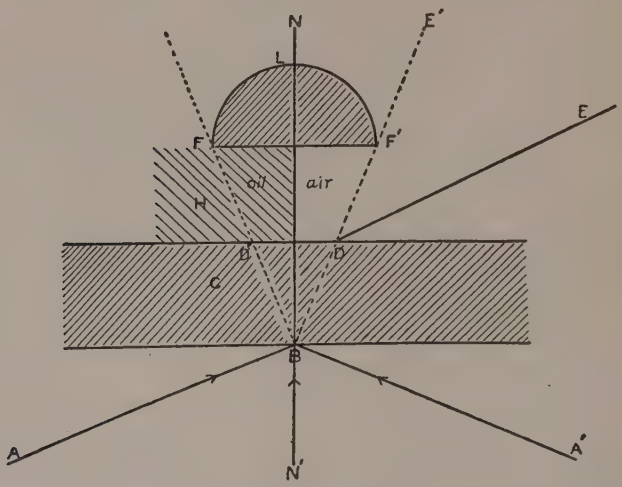

FIG. 49.—DIAGRAMMATIC REPRESENTATION OF THE OPTICS OF THE OIL IMMERSION LENS.

many purposes the lower powers of the compound microscope may be used, but in a critical study of the individual bacterial cells it is customary to employ the highest power, or the homogeneous oil immersion objective.

The Oil Immersion Objective.—Even a superficial examination of the various objectives used on compound micro-

scopes will show that in general the higher the power, that is, the greater the magnification, the smaller is the opening through which the light must pass at the lower end of the objective. It is evident that with the higher powers the amount of light which may enter the lens is considerably reduced. It has been found in practice that the interposition of a drop of oil between the lens and the object to be examined make a far more satisfactory definition, the object being seen much more clearly. Inasmuch as oil must always be used with the highest power lens, and should never be used with other lenses, a brief explanation of the reasons for its use will be given.

The diagram in figure 49 should be consulted. Let C represent the glass cover slip over the object to be examined, and L the lens through which light must pass to the eye of the observer. The diagram shows a drop of oil, H, lying to the left of the line NB. This oil is usually oil of cedar wood, having the same refractive index as the glass used in the slide. By means of the mirror and the substage condenser the rays of light are focused upon the object which is to be examined. These rays of light may be represented by AB and A'B. Rays of light which are normal, that is, perpendicular to the surface of C, such as the ray BN, will pass straight through without refraction in glass, air, or oil. A ray such as AB, however, upon entering the denser medium, glass, will be refracted in the path BD. Here it enters the air and is again refracted in a direction parallel to the incident ray, that is, in the direction of DE. If, however, a drop of oil replaces the air this second refraction would not occur. This is illustrated by following the ray A'B which is refracted in the glass as BD'. Upon entering the oil it continues in the same direction as the ray BF. It is evident that all rays lying between A'B and BN and N'N will enter the lens L when oil is present, but that only a portion will enter if air is between the lens and the

cover glass. Evidently the light entering the lens is more concentrated when oil is used. This results in a more brilliantly illuminated field and more satisfactory observation.

Microscopic Observation of Colonies and Cultures.—Bacteria, yeasts, or molds, growing on agar or gelatin plates are advantageously observed under the low power of the microscope, either by inverting the plate and examining the colony through the medium, or by removing the cover and observing directly. This is particularly advantageous in studying molds. Frequently the arrangement of spores on conidiophores and the manner in which conidiophores branch can be better determined in this fashion than by mounting material under a cover glass. Observation of bacterial colonies under the lower power will frequently show structural peculiarities which may be useful in differentiating the related species from each other.

Unstained Preparations.—Microörganisms are not infrequently examined in the living condition by placing them in a drop of suitable liquid such as physiological salt solution, water, or broth on a slide and covering them with a cover glass. Such preparations are made for the purpose of observing the appearance of the living organism, also to determine motility, and in some cases to study method and rate of growth. It is sometimes difficult to mount molds in this manner without numerous air bubbles interfering with satisfactory observation. This difficulty may be obviated by a drop of alcohol followed by a drop of water.

Most microörganisms (bacteria in particular) are comparatively transparent and can be seen under the microscope unstained only because their refractive index is different from that of water. Considerable care is, therefore, frequently necessary in the adjustment of the light, by shifting the mirror and the iris diaphragm of the substage, or of the Abbé condenser.

Stained Preparations.—It is comparatively difficult to observe unstained bacteria satisfactorily. The details of the morphology of the molds and of the yeasts are also better indicated in stained preparation. Some of the morphologic characters cannot be seen at all except by the use of an appropriate stain.

The stains commonly used in the bacteriological laboratory are certain of the so-called aniline dyes. These are usually divided into two groups, the basic dyes, and the acid dyes. In staining the cells of higher plants and animals it will usually be observed that the basic dyes stain such objects as the chromatin material in the nucleus, while the acid dyes more commonly stain the cytoplasm of the cell. Most bacteria stain best by the use of the basic dyes. Among these are gentian violet, methylene blue, and basic fuchsin. For special purposes, and for use as contrast stains, acid dyes are sometimes used, particularly Bismarck brown, eosin, and safranine. These stains are usually employed in aqueous solutions. Methods of preparation of the particular stains used may be found in laboratory manuals.

Substances not in themselves true dyes are sometimes of considerable significance in staining. When cells are treated with certain compounds such as aniline oil, iodine, or iron tannate, the ability of certain parts of the cell or certain cell structures to take up stain may be greatly increased. Substances thus used to increase the staining power of an organism are called *mordants*. For example, the flagella of bacteria are in general so slender and so difficult to stain that they are not to be seen in ordinary preparations. However, when the bacteria are suitably mordanted with iron tannate the flagella may then take up the stain in sufficient amount as to make them readily visible.

In the preparation of any stained mount the following

steps are usually necessary: spreading, drying, fixing, staining, washing, and mounting. A small amount of the organism to be studied, usually bacteria or yeasts, may be mixed in a drop of water on a clean cover-glass and spread over the surface in a uniform layer. The glass should be sufficiently clean and free from grease or oil so that there will be no tendency for the water to round up in the form of a drop. If the organism to be studied has been growing in a liquid medium, such as broth, the water may not be necessary, but it may be spread directly in its nutrient medium upon the cover glass. After spreading, the material is allowed to dry in the air, or it may be gently heated, using care that the organisms are not overheated. The preparation is then fixed by passing it rapidly two or three times through the flame of the Bunsen burner. This hardens the protoplasm of the organisms and causes them to stick firmly to the glass, so firmly in fact that they may be washed vigorously without being loosened. A drop of the stain to be used is then spread over the surface, allowed to act for the desired length of time, then washed off by means of the tap. The cover glass may then be dried and inverted over a drop of water or a drop of balsam on a glass slide and is then ready for microscopic observation.

In many laboratories the cover glass is dispensed with, the mount being made directly upon the glass slip or slide. When dried after staining, the immersion oil is placed directly upon the mount and examination made without the interposition of the cover glass.

For staining particular parts of the cell, and for demonstrating peculiarities of staining reactions, certain special methods have been devised. Of these, four are of sufficient importance to deserve comment in this connection. They are the spore stain, the stain for acid fast bacteria, the Gram stain, and the stain for flagella.

Spore Stain.—The spores of yeasts and of bacteria are not readily penetrated by stains, but when they are stained they are somewhat more resistent to decolorization than the vegetative cells; this makes possible a staining method which will show spores of one color and vegetative cells of another. The material to be examined is spread and fixed in the manner already described. It is then flooded with some powerful stain, usually carbol fuchsin. The mount is then heated until the carbol fuchsin steams for a period of five minutes. Care must be used to replace stain with fresh as rapidly as it evaporates so that the mount does not become dry over any part. The excess stain is washed off with water, then with dilute (usually 5 per cent) acetic acid, until the mount is light pink in color. Usually a few seconds will be sufficient. It is then thoroughly washed in water to remove all traces of acid. This procedure leaves the spores red but the vegetative rods colorless. The latter are stained by adding some Loeffler's methylene blue. When this has acted for a few seconds the mount is washed, dried, and examined. The spores will ordinarily appear red and vegetative cells blue. With the yeasts the spores will appear red and the asci blue.

Study of Acid Fast Bacteria.—A few species of bacteria, some of them of considerable economic importance, such as the organism causing tuberculosis, are said to be *acid fast* or *acid proof*. Like bacterial spores they resist the penetration of stains, but when once the stain has penetrated they are not easily decolorized, in fact, they will withstand the decolorizing action of strong acids or alcohol for a considerable length of time. The method of procedure is essentially the same as in staining spores, except that as a decolorizing agent a strong solution of acid, usually sulphuric acid, is used. Other bacteria or materials present may be counterstained by methylene blue. The acid fast bacteria appear as red rods in a blue field.

Gram's Stain.—Gram's staining method is one very frequently used for the differentiation of bacteria. It was originally used for the demonstration of bacteria in tissues. Essentially it consists in staining bacteria with one of the aniline dyes, usually gentian violet or thionin, adding a solution of iodine as a mordant, then decolorizing with alcohol. Bacteria may be divided into two groups, those which are Gram-positive, that is, retain the color, and those which are Gram-negative.

Staining of Flagella.—It was noted above that the flagella of bacteria are usually so slender and so difficult to stain that they are not seen in the ordinary stained preparation. Many methods of mordanting the flagella so that they will stain more readily have been suggested and used in practice. One of the most common methods is to use iron tannate as a mordant, followed by carbol fuchsin as a stain. It is important in making such preparation that only young actively motile cells be used. It is best not to employ cultures more than twelve to eighteen hours old.

SECTION III

PHYSIOLOGY OF MICROÖRGANISMS

CHAPTER IX

EFFECT OF PHYSICAL ENVIRONMENT UPON MICROÖRGANISMS

Bacteria as well as all other microörganisms are constantly being subjected to physical and chemical environment which bring about increases or decreases in the rates of growth, or increases and decreases in the rates of death, and various alterations in morphology, physiology and cultural characters. Before discussing these effects it is necessary to note the laws which govern rates of bacterial multiplication and rates of death.

Rates of Growth and Death

With large plants and animals it is comparatively easy to determine rates of growth by weighing at suitable intervals. With organisms like bacteria and yeasts, however, it is usually best to count the number of living cells present after varying lengths of time. It has already been noted that bacteria multiply by fission, that is, each mother cell divides into two daughter cells. The length of time which elapses between consecutive cell divisions, that is, the length of time that is required for a single cell to grow to its full size, and divide to form two individuals, is called the *generation time*. It is apparent that the shorter the generation time the more rapidly are the bacteria multiplying. We can, therefore, judge of the effect of various physical or chemical influences upon the growth of an organism by noting any variations in the length of the generation time which are produced. It is usually not convenient actually to watch the microörganisms under the microscope and to

determine by means of a stop watch the length of time required for cell division or the length of the generation time. Methods of counting, however, have been devised so that we may know the number of living bacteria present in a cubic centimeter of liquid at the beginning of any definite period of time and the number at the end of that period. From this it is possible to calculate the length of the generation time as follows: let n be the number of cell divisions, that is, the number of generations which develop during a given time, which we may represent by t. If we start with one organism we shall have at the end of one generation period two organisms, and at the end of the second generation period four organisms, and at the end of third period eight organisms. It is apparent, therefore, that the number of organisms originating from a single cell will be at the end of the first period 2^1, at the end of the second 2^2, at the end of third period 2^3, and at the end of the nth period 2^n. If instead of beginning with a single organism we start with any number, which we may represent by B, the number at the end of the nth generation period will be

$$B\ 2^n$$

If we let the number of bacteria after time t be represented by b we have the equation

$$b = B\ 2^n$$

If this equation is solved for n it will be found that

$$n = \frac{\log b - \log B}{\log 2}$$

It is evident that the number of generations which will develop in time t will be equal to the total time divided by the generation time, that is,

$$n = \frac{t}{g}$$

or conversely

$$g = \frac{t}{n}$$

By use of these formulae for determining the value of g in any growing culture of microörganisms we may detect the effect of changes in environment. Within certain limits, increasing the temperature at which the culture is kept will increase the rate of growth, that is, g will diminish, and the smaller g is, the more favorable are the conditions. Conversely, the larger g is, the more unfavorable are the conditions.

When organisms are placed under sufficiently unfavorable conditions they cease to multiply and begin to die instead. In most of the cases which have been carefully studied the bacteria die off in accordance with a definite law, which may be stated as follows: with a given kind of organism under uniform conditions, the number of bacteria present in a culture will always be reduced by one-half in equal periods of time, that is, no matter how many bacteria there are at the beginning of a definite period of time, one-half that number will always be alive at the end of the proper interval. For example, suppose that two cultures, one containing a million bacteria and the other a thousand bacteria are subjected to the same unfavorable conditions. It is found that at the end of a definite period of time, say ten minutes, the bacteria in the less concentrated suspension average five hundred. It will be found that in the same perod of time the other culture has also been halved, that is, there are 500,000 bacteria left. Another way of stating is this: during each equal interval of time a definite percentage of those bacteria living at the beginning of the period will be killed. If we wish to compare unfavorable conditions in their effect upon the death of microörganisms we may compare the length of time required to reduce the

numbers of bacteria by a definite percentage, say one half. If at one temperature, for example, half of the bacteria are killed in ten minutes, and at another temperature one half are killed in five minutes, it is evident that the second temperature is far more destructive than the first. It will be noted that the time required to kill half the bacteria is mathematically the converse of the generation time.

In summary, it may be emphasized that all effects of environment upon microörganisms may be manifested in growing cultures by changes in the length of the generation time, changes in morphology, and in the physiologic and cultural reactions. Likewise the effect of environment upon the rate of death of bacteria may be noted by comparing the length of time necessary to kill a definite percentage of the microörganisms present.

In the present chapter the effect of the physical environment will be discussed. The most important of the physical agencies are light, heat, electricity, the radiations from Röntgen tubes and from radium, pressure, osmotic pressure and desiccation.

Effect of Light

Light affects organisms in several ways. Certain species of bacteria, particularly those which contain a purple coloring matter known as bacteriopurpurin, are attracted by light; when motile they swim in the direction of the light source. When growing, for example, in a suitable medium illuminated from one side, they will move toward the brighter side and collect on the surface of the glass. The effect of any agency upon the direction of movement of an organism is termed a *taxy*. The power of light to attract is termed *phototaxis*. Most of the bacteria, however, do not grow well in the presence of light; in fact, light exerts a destructive influence.

Light in general interferes with the growth of micro-

örganisms, and if too intense stops their growth and accelerates the death rate.

Light sometimes affects the direction of growth of microörganisms, particularly molds. The fertile hyphae, that is, the conidiophores or sporangiophores of certain species of molds tend to bend in the direction of light. An effect of any external agency upon the direction of growth is called a *tropism;* this effect produced by light is therefore termed *phototropism.*

Living protoplasm in general is more or less injuriously affected by light. Too intense light applied to the human skin, for example, will cause sunburn. Green plants accustomed to growing in the shade will likewise be sunburned when exposed to direct sunlight. Certain light rays have the power of causing coagulation of proteins. It is not surprising, therefore, that microörganisms which do not require light for their development are particularly sensitive to it, and may be rapidly destroyed by certain rays.

It will be recalled that sunlight, that is, white light, may be divided into various rays of different wave lengths. At one end of the visible spectrum are the reds, at the other end the blue and the violet. Beyond the violet with wave lengths still shorter is the so-called ultraviolet. In general the rays of light in the blue and violet and in the ultraviolet are particularly destructive to bacteria. It may be observed that these are the same rays which also affect most intensely the photographic plate.

Commercial use of ultra-violet light in sterilization has been attempted. The most satisfactory source of such light is the mercury vapor arc lamp, or so-called Cooper-Hewitt lamp. This is a quartz, not glass (glass is opaque to ultraviolet light) cylinder from which most of the air has been exhausted and which contains a small amount of mercury. This mercury is vaporized and an arc passes from one end to the other, causing the entire tube to glow. The light

given off is largely blue, violet and ultra-violet. Relatively short exposures of living cells to this light will kill them.

Effect of Heat

For every organism there exists a group of temperature relationships. The lowest temperature at which any appreciable growth will develop is called an organism's *minimum* temperature. That temperature at which it grows most rapidly is its *optimum*. The highest temperature to which it will grow is its *maximum*. The number of degrees difference between the maximum and minimum growth temperatures is termed the *growth temperature range*. An additional temperature relationship has been termed the *thermal death point*.

The Minimum Temperature.—As the medium in which an organism is growing is cooled, in general the generation time increases until it reaches a point where there is practically no multiplication; below this temperature there will be no growth and the bacteria will die off more or less rapidly, depending upon circumstances. If other conditions are constant, the colder the microörganisms, the less slowly do they die. Dried bacteria, for example, may be exposed to the temperature of liquid air without being killed.

The minimum growth temperature varies greatly with different bacteria. Some microöganisms can multiply at temperatures below 0° C. Such multiplication, for example, has been observed in butter and in brine in cold storage.

Optimum Growth Temperature.—As the temperature of a culture is increased from the minimum the generation time of the microörganisms will decrease in length, that is, the bacteria will multiply more and more rapidly. This continues with increase in temperature to a certain point; heating beyond this will then again increase the generation time, that is, slow down the growth rate. That tempera-

ture at which the generation time is the least or that temperature at which the organisms are multiplying most rapidly is the optimum.

Bacteria are sometimes divided into several groups using the optimum temperature as the basis for classification. Those bacteria which grow best at relatively low temperatures, such as the temperature of the ice box, are termed *psychrophilic.* Such bacteria are found in cold water, or in lakes, the arctic regions, etc. Not many of them are of economic importance. Those bacteria which prefer to grow at moderate temperatures, such as room temperature or blood temperature, are termed *mesophilic.* The mesophilic bacteria may be again subdivided into those which grow best at temperatures of 20-25°, that is, at ordinary room temperature, and those which grow best at blood heat at 37.5°. Among the organisms belonging to the latter group are those which are the disease producers in man and animals. A few bacteria are known which have optimum temperatures decidedly above blood heat, that is, above 37.5° Some species of bacteria will grow only at temperatures of 45° to 60°. They are termed *thermophilic.* Such organisms are not uncommon in the soil, and are of significance in food canning.

The Maximum Temperature.—As the temperature is increased above the optimum, the generation time of bacteria increases, that is, the rate of growth decreases, until at last it comes practically to a standstill. The highest temperature at which there is appreciable growth is termed the maximum. Here again much variation may be found among microörganisms. Certain of the psychrophilic forms will not grow at temperatures above that of the room. Many of the pathogenic bacteria have maximum temperatures between 40° and 45° C. Certain of the thermophilic bacteria will continue to grow at comparatively high temperatures, even 65° to 70°, that is, they will grow at a

temperature which will cause slow coagulation or cooking of egg white.

Growth Temperature Range.—Some bacteria have a very wide growth temperature range; for example, the *Bacterium coli* will grow slowly at both 12° and 45° C., giving a growth temperature range of at least 33°. Other bacteria, such as some of the pathogenic forms, may have maximum temperature at 40° and minimum temperature at 35°, giving a growth temperature range of only 5°. It is apparent that for such bacteria the temperature at which they are grown must be carefully controlled if successful results are to be secured.

Thermal Death Point.—While the term thermal death point is commonly used in literature, it is not entirely satisfactory to express the particular idea. A thermal death point is sometimes defined as that temperature which in a given length of time will kill a particular organism. We have already seen that all the organisms in a culture do not die instantaneously upon being subjected to unfavorable conditions, but that under a definite set of conditions, there will be a definite rate of death. Theoretically it would be better to designate the rate of death under certain standard conditions at a definite temperature. The rate at which bacteria die off at a given temperature is influenced by several factors. Bacteria which *produce spores* are frequently said to have two thermal death points, one for the vegetative rod and another for the spores. The latter are much more difficult to kill, that is, they die off less rapidly under unfavorable conditions than do the vegetative cells. The hydrogen ion concentration of the medium also is important. The higher the *hydrogen ion concentration* or the higher the *hydroxyl ion concentration,* the more rapidly will bacteria die off at a given temperature. The *presence or absence of moisture* may also be important. Bacteria that are not killed by drying are much more resistant in the

dried condition than the same organisms when moist. It has already been noted that steam is more effective in sterilization than is dry heat. If the thermal death point is to be determined it should be defined as that temperature which in a given period of time will destroy all of a definite number of bacteria, noting particularly the composition of the medium and its hydrogen ion concentration. Another method of determination is to give under definite conditions the length of time required at a definite temperature to reduce the number of bacteria by one half.

Effect of Electricity

Bacteria are usually little affected directly by the passage of electricity. However, the passage of the electric current may cause electrolysis and the consequent formation of substances which act as chemical disinfectants. When a current of sufficient strength is passed through a solution containing sodium chloride, for example, chlorine gas appears at one electrode and alkali at the other. The free chlorine and hypochlorites which are formed act as powerful disinfectants. This method has been utilized to some extent in the sterilization of sewage and water.

Effect of X-Rays and Radium Rays

The rays given off by the Röntgen tube and by radium have been studied in their bactericidal effect, but they have not proved to be sufficiently powerful to be of economic importance.

Effect of Pressure

Bacteria are in general resistant to relatively high pressures. In one series of experiments a pressure of three thousand atmospheres (an atmosphere is about 15 pounds per square inch) was shown not to kill the *Bacterium typhi, Bacterium coli,* or many other species of bacteria. A

pressure of six thousand atmospheres continued for fourteen hours was found to destroy all non-spore-forming organisms. The spores of bacteria are not uniformly and regularly killed even at pressures of twelve thousand atmospheres. The pressures required to kill bacteria are so enormous that they have not been found practicable in the sterilization of food materials. In general bacteria that are killed by high pressure are found to be difficult to stain, only shadows being discernible. Gram-positive bacteria in general lose their specific staining reaction.

Effect of Osmotic Pressure

It will be recalled that the protoplasm of all cells is bounded by a semipermeable membrane termed the ectoplast. Some substances in solution can pass through this, others cannot. Water usually penetrates it readily. Whenever the concentration of solutes on the interior is such that osmotic pressure is greater on the inside of the cell than on the outside, water passes in and the cell becomes turgid. This is the normal condition of cells. When a cell is plunged into a concentrated solution of some substance which will not pass through ectoplast, the water passes from the inside of the cell to the exterior, the protoplasm tends to shrink, and the cell is said to have undergone *plasmolysis*. On the other hand, when an organism has become accustomed to growing in concentrated solutions, such as syrups, and is then dropped into distilled water the pressure on the interior of the cell is much greater than on the exterior. The cell will then tend to swell and perhaps burst. This condition is termed *plasmoptysis*. This latter phenomenon may be readily observed by placing a drop of distilled water over the tips of the hyphae at the margin of a mold colony growing upon beer-wort agar. The distilled water suddenly diminishes this concentration locally, the pressure on the interior of the cell being such

that the tip is forced open and the protoplasm is squeezed out much as tooth paste may be forced out from a collapsible tube.

By suitably increasing the concentration of solutes the point may be reached beyond which an organism cannot adapt itself. Such a concentrated solution then acts as a preservative. This is the important factor in preserving such foods as syrups, jellies, and jams.

Solutions which have equal osmotic pressures are said to be *isotonic*. Solutions are isotonic for a cell when they will cause neither shrinking nor swelling of the cell contents. The most common of the isotonic solutions used in the laboratory is the so-called *physiological salt solution*, containing usually 0.85 per cent sodium chloride. This concentration of sodium chloride is such that it is isotonic with the red blood cells of the blood. It is also isotonic with the blood serum, consequently red blood corpuscles dropped into such solution neither shrink nor swell.

Effect of Desiccation

Most yeasts and bacteria grow best in the presence of an abundance of moisture. Complete *desiccation*, that is, drying, will cause many kinds of bacteria to die off relatively rapidly. Other species, particularly the spore formers, are more resistant to the drying and they die off much more slowly. Some bacteria that do not produce spores and many of the yeasts are also relatively resistant to drying.

The reasons why drying should be so destructive to certain kinds of bacteria are not easily determined. Undoubtedly in some cases the death of the cell is due to the unusual concentration of solutes about the organisms when it is drying, and to the consequent increase in the osmotic pressure. It has been found that certain microörganisms if frozen in a vacuum and dried quickly, are not killed.

Evidently the freezing prevents the concentration of the solute with increased osmotic pressure. Drying, of course, will prevent any growth of microörganisms, so that this method is used extensively in the preservation of foods.

CHAPTER X

PHYSICAL EFFECTS PRODUCED BY MICROÖRGANISMS

Practically all of the chemical changes brought about by microörganisms result in physical changes becoming apparent in the medium in which the organisms have been growing. There are four of these changes, however, distinctly physical in nature that are worthy of notice. These are: the production of light, the development of heat, changes in viscosity of medium, and changes in osmotic pressure.

Light Production by Microörganisms

The property of producing light, that is, of being *photogenic* or phosphorescent, is one which is common to plants and animals of a great many different types. A number of species of bacteria have been described as photogenic. Practically all of them have been found in sea water or upon food materials, such as salt fish, taken from the sea. These bacteria may be grown readily upon suitable culture media, particularly media containing proper amounts of salts. When grown upon an agar slant, for example, distinct light may be observed when the culture is examined in a dark room. Sufficient light may be given off so that the organisms may be photographed by their own light. The bacteria which are found to be phosphorescent or photogenic are all aërobes, growing well only in the presence of oxygen and not producing light in its absence. The phenomenon of photogenesis in bacteria has not been adequately studied, but there is no reason to suppose that it differs materially from the same phenomenon observed in higher

plants and animals. In these forms it has been found possible to extract by suitable methods from the phosphorescent organs, two substances which when mixed will cause a solution to glow. The light developed is rather yellowish-green in color. When these organisms occur in great numbers, as they may occasionally in sea water, they may be the cause of diffused phosphorescence. They sometimes produce luminescence on decaying fish that have been thrown up by the waves on the seashore.

Production of Heat

Microörganisms in general secure their energy for development and growth by oxidative processes. In most instances some of the energy secured is released in the form of heat. Such heat production may be studied by use of Dewar flasks, which are especially designed to conserve heat and prevent its radiation. By this use it is usually possible to measure the amount of heat developed by various fermenting materials, such as by yeast in cane sugar, or by bacteria in the souring of milk. The latter phenomenon has been studied by Hill (1911). This author found that in a little less than twenty-four hours the bacteria produced approximately one and three-tenths calories of heat per gram of milk, during the process of souring.

Bacteria in nature sometimes produce appreciable increases in temperature where there is opportunity for oxidation, and the heat is not rapidly radiated. Loosely piled manure, for example, or damp straw will heat, the temperature rising in some cases to 60° or 70° C. The fact that heat is gradually given off during the process of decomposition is made use of by the market gardeners in the preparation of hot beds. The amount of heat given off by the manure used is sufficient to raise appreciably the temperature under the glass frame. Loosely packed ensilage may also show some rise in temperature, and frequently

there is a very marked increase within a few feet of the top of the silo where air may have access to the material. The heat of oxidation is also made use of in certain methods of curing hay in which it is allowed to heat in piles, and is then spread and dried rapidly. It is possible by this means to secure a palatable product in countries where the climate is too moist to dry the hay in the ordinary manner.

Changes in Viscosity

Some organisms may markedly increase, others decrease the viscosity of solutions in which they are growing, as will be discussed in greater detail in a later chapter. Many species of bacteria are known to produce gums. The accumulations of these gummy or mucilaginous materials in the solution in which the organism is growing will increase materially its viscosity. For example, certain bacteria growing in milk may cause it to become ropy or stringy. It is possible that in some cases the increase in viscosity is due to partial digestion or hydrolysis of protein material. Under certain conditions bread may become viscous or ropy as the result of the growth of certain putrefactive bacteria. Apparently the most marked change in this case is the transformation of the gluten.

Conversely some bacteria are markedly capable of reducing the viscosity of the medium in which they are grown. Certain bacteria digest gelatin and thus decrease its viscosity. Other organisms are able to soften starch paste, or to liquefy coagulated blood serum; a few species can even digest agar-agar.

Effect on Osmotic Pressure

The osmotic pressure of any solution varies directly with the total number of ions and molecules dissolved. Consequently when complex molecules are broken down into simple molecules there is a resultant increase in the osmotic

pressure, for example, when proteins are broken down through peptones to amico acids, there is marked increase in osmotic pressure. When starch, which has almost no osmotic pressure, is broken down into dextrins, maltose, and finally to dextrose there is relatively an enormous increase in osmotic pressure. Bacteria when growing in the tissues in the body may bring about changes which increase the osmotic pressure and tend in consequence to abstract water from the tissues.

Conversely, when simple molecules are combined with the more complex there is a resultant decrease in the osmotic pressure. Certain bacteria polymerize sugars into polysaccharides, the latter having practically no osmotic pressure.

CHAPTER XI

EFFECT OF CHEMICAL ENVIRONMENT UPON MICROÖRGANISMS

THERE are four distinct and relatively important methods by which the chemical environment may influence microörganisms: motile bacteria may be influenced in their *direction of movement*, molds and filamentous bacteria may be influenced in their *direction of growth*, all types of microörganisms may be influenced as to their *rapidity of growth*, and as to their *rate of death* under unfavorable conditions.

EFFECT OF CHEMICALS ON DIRECTION OF MOVEMENT

It has previously been stated (Chapter IX) that the effect of any external agency upon the direction of movement of an organism is termed a *taxy*. When the agent influencing such direction of movement is a chemical, the phenomenon is termed *chemotaxis*.

Chemotaxis may be most readily demonstrated by immersing the tip of a fine capillary glass tube partly filled with a solution of beef extract or peptone, in a drop of water containing numerous motile bacteria. Within a short time most of the bacteria in the drop will be found moving about the tip of the tube, and many will have entered. The chemical in solution has been passing, by diffusion, out through the water. The bacteria apparently tend to swim in the direction of the greater concentration. Such a phenomenon of attraction may be termed *positive* chemotaxis. Some chemicals may exert the reverse influence, that is *negative* chemotaxis. In general

bacteria are repulsed by solutions containing alcohol or strong acids.

The white blood corpuscles of the blood are attracted under certain conditions to the invading bacteria. The cells have some power of independent motion. This positive chemotaxis toward bacteria is one of the most important items in the list of body defenses against disease.

Some bacteria are markedly attracted by oxygen, a phenomenon termed *aërotaxis*. If a drop of sewage swarming with bacteria be mounted under a cover glass on a glass slide, using care to include a few small air bubbles, within a short period of time the microörganisms, both bacteria and protozoa, will be found to arrange themselves in quite regular concentric lines about these bubbles. Apparently each motile species influenced by oxygen concentration seeks to remain within that zone where the oxygen concentration is most favorable. This fact has been put to use by bacteriologists in isolating certain species of bacteria. For example, the organism which causes Asiatic cholera may be detected in feces by adding a small amount of the stool to broth. The many kinds of bacteria present will multiply but the cholera vibrio is attracted most by oxygen and will be present, therefore, in relatively much larger numbers in the surface layer. An examination of a drop from the surface will show the vibrios to be numerous if the test is positive.

Effect of Chemicals on Direction of Growth

The effect of an external agency upon the direction of growth has already been defined as a *tropism*. The effect of a chemical is correspondingly termed a *chemotropism*. That type of chemotropism most frequently observed is the attraction or repulsion exerted by water, termed *hydrotropism*. Most species of mold show this to a marked degree. Those hyphæ which are differentiated for spore production,

that is, the fertile hyphæ, show in general a negative hydrotropism. This is the reason why the conidiophores and sporangiophores of the various molds generally rise at right angles to the surface of the medium upon which the organism is growing. As a result the spores are developed in an environment relatively dry, and the conditions are, therefore, much more favorable for their distribution by currents of air. It will be noted that hydrotropism is much more important in controlling the direction of growth of molds than it is in higher plants. For the latter, gravity is frequently the agency most active in determining growth direction.

Effect of Chemicals on Rapidity of Growth

Various chemicals exert a marked influence upon the rapidity of growth of microörganisms, that is, upon the length of the average generation time. Among the more important of these may be mentioned the concentration of hydrogen ions, the concentration of oxygen, the concentration of nutrient substances, the kinds of nutrient substances present, the stimulating effect of dilute poisons, and the effect of substances of autogenic origin not belonging to any of the preceding groups.

Effect of Hydrogen Ion Concentration on Rapidity of Growth.—For every organism there exists that concentration of hydrogen ions in the medium which is most favorable for development, that is, the *optimum;* likewise a *maximum* concentration beyond which it cannot grow; and a *minimum* concentration, that is, a concentration of hydroxyl ions likewise in which it cannot develop. The growth limits vary widely with different species. Most species of bacteria prefer to grow in hydrogen ion concentrations represented by the P_H values 7 to 8. Yeasts and certain species of molds prefer solutions which are somewhat more acid. It has been found by laboratory tests that

moderate changes in hydrogen ion concentration do not markedly change the rate at which bacteria grow except near the limits. Practically the optimum is a relatively wide range. It has previously been emphasized that in the preparation of certain types of media great care is necessary to insure the correct hydrogen ion concentration; otherwise growth will not occur.

Effect of Oxygen upon Rapidity of Growth.—Apparently for each kind of organism under a definite set of conditions or in a particular culture medium, there exists a certain concentration of oxygen which is most favorable to growth. This may be termed the *optimum*. For most bacteria there likewise exists a point in diminished oxygen pressure where the concentration is not sufficient to allow of growth. This may be termed the *minimum*. For certain bacteria there likewise exists a *maximum* concentration, and the organisms will not grow in a medium containing more than this amount. Bacteria which require a relatively high concentration of oxygen, usually about that of the atmosphere, are said to be *aërobic*. For such bacteria the optimum and the minimum are particularly significant. These bacteria will grow usually only on the surface of media or in the surface layers. When inoculated into a fermentation tube, for example, they will grow only in the open arm near the surface of the medium. At the other extreme there are bacteria whose optimum condition apparently is approximately complete absence of free atmospheric oxygen. Many of these will not develop at all in an atmospheric concentration. Such organisms are termed *obligate anaërobes*. For these anaërobic bacteria the optimum and the maximum concentrations of oxygen are most important. Lying between these two extremes are certain bacteria termed *micro-aërophiles*, which grow best in a concentration of oxygen something less than that of the atmosphere, but

do not grow in the entire absence of oxygen. In a suitable medium such organisms will develop in a definite layer some distance below the surface. Still other bacteria are termed *facultative.* These organisms apparently will grow either in the presence of atmospheric oxygen or in its absence. In practically every instance, however, they grow in the absence of oxygen only when there is nitrate or some sugar or other available carbohydrate present. The *Bacterium coli,* for example, will grow only in the open arm of the fermentation tube in broth, but when sugar is added it will grow equally well in the closed arm. It is possible that the oxygen relationships of some microörganisms are more complex than indicated. It is stated that the organism of contagious abortion in cattle has two oxygen optimums, it will grow either in decreased oxygen concentration or in greatly increased oxygen concentration, but not well in the presence of atmospheric concentration except after long continued cultivation.

The fact that many organisms will not grow in the presence of free oxygen makes it necessary to use special methods of cultivation in the laboratory. Media in which anaërobic bacteria are to be cultivated may be boiled just before use in order to drive out the oxygen. After inoculation they may be covered by a layer of oil or paraffin, or the air may be displaced in closed tubes by some other gas. Most frequently hydrogen is used or oxygen is absorbed by use of some reagent such as sodium pyrogallate, leaving an atmosphere of nitrogen. In some cases a vacuum may be used satisfactorily.

Effect of Kinds of Nutrients Present.—By careful experimentation it is usually possible to find that combination of nutrient materials which will allow of the most rapid development of a particular microörganism. The substances needed and the proportions vary greatly with

the microörganism. Some forms can utilize inorganic materials exclusively, others require the most complex of organic compounds for their best growth.

Effect of Concentration of Nutrients.—For each nutrient there must be an optimum concentration at which the microörganisms will grow most rapidly. An increase in concentration beyond this point will more or less rapidly cut down the rate of growth, that is increase the length of the generation time.

Stimulating Effects of Dilute Poisons.—Substances which are ordinarily regarded as distinctly poisonous to microörganisms, may when sufficiently dilute exert a stimulating effect. Certain of the aniline dyes, for example, when present in sufficient concentration prevent all growth of microörganisms, but when greatly diluted they may increase the rate of growth.

Effect of Autogenic Substances.—Practically all microorganisms more or less rapidly produce substances inimical to their own development. In some instances the number of bacteria which develop in a culture medium is limited by the lack of some particular food constituent. More frequently in the laboratory it is limited by the accumulation of these toxic substances. Just what these are is not known in most cases. Apparently in some instances they are destroyed by heat and may be removed by filtration through porcelain filters. As these substances accumulate, the rate of development decreases, the generation time increases, until finally the bacteria are dying off more rapidly than they multiply.

Effect of Chemicals upon Rate of Death

In any culture or mass of bacteria in which there is no multiplication the cells are dying. The death rate may be very small, but the bacteria are, nevertheless, decreasing in number.

An *antiseptic* is any substance which will inhibit the growth of microörganisms. It is, of course, true that if growth is prevented the cells will die off more or less rapidly. A substance which causes comparatively rapid death is termed a *germicide.* It will be apparent that the difference between antiseptic and germicide is quantitative and not qualitative. Both kill bacteria, antiseptics slowly, germicides rapidly.

The term *disinfectant* is for practical purposes synonymous with germicide. It is usually used with reference to disease-producing or pathogenic bacteria.

A *preservative* is an antiseptic substance which is added to foods to prevent the growth of harmful microörganisms. The term *fumigant* is sometimes applied to a gaseous disinfectant. A *deodorant* is a substance which will mask or destroy obnoxious odors; it is not necessarily a fumigant or a disinfectant. A substance which will kill bacteria is said to exert a *bactericidal* action.

For convenience in discussion, substances capable of destroying bacteria may be divided into three groups: first those which will kill bacteria free in nature; second, those which may be injected into or absorbed by the body of man or animal and will destroy microörganisms growing there; third, those substances capable of killing microörganisms which are produced by animals and are present in the blood serum.

The germicidal agents commonly used in the destruction of microörganisms may be grouped under the headings of *electrolytes,* including the *acids, alkalies,* and *salts;* the nonelectrolytes, including the *alcohols, aldehydes, anæsthetics,* the *phenol derivatives,* the *essential oils;* and lastly the oxidizing agents, including *oxygen, ozone, chlorine,* and similar materials.

Characteristics of an Ideal Disinfectant.—Certain characteristics should be possessed by an ideal disinfectant. To

the extent that a particular disinfectant measures up in its characteristics to those of the ideal disinfectant, it is valuable for general use. The important characteristics of disinfectants are as follows:

1. *High Germicidal Power.*—The ability of a particular disinfectant to kill microörganisms is usually compared with that of phenol. In making such comparisons it is customary to use the *Bacterium typhosum*, the cause of typhoid fever, as the test organism. A series of test tubes are prepared containing varying dilutions respectively of phenol and of disinfectant to be tested. Equal numbers of *Bacterium typhosum* are then introduced into each tube. At intervals of two and one-half minutes samples are taken from each tube and transferred to a nutrient medium suitable for growth. The transfers are continued for fifteen minutes. The strength of phenol which is required to kill the organisms, that is, so that no growth is secured when a loopful is transferred to sterile broth after two and one-half minutes exposure, is determined, likewise the strength of the disinfectant to be tested which will produce the same results. The ratio between the dilutions of the disinfectant to be tested and the phenol is determined and the number recorded. The ratio between the concentration of the phenol and of the test disinfectant required to kill in fifteen minutes is also determined. These two ratios are averaged. For example, if phenol kills in two and one-half minutes in a strength of one to one hundred, and the disinfectant to be tested in one to five hundred, the first ratio would be five. If in fifteen minutes phenol kills in one to two hundred and the disinfectant to be tested in one to twelve hundred the ratio would be six. The average of the two would be five and one-half. Most commercial disinfectants, particularly the coal tar products, are sold upon the basis of their *phenol coefficient.*

2. *Stability.*—The disinfectant, to be most valuable, should be relatively stable in the presence of organic matter. Some of the most powerful of the disinfectants combine with organic matter forming insoluble compounds and pass out of solution relatively completely. The strength of the disinfectant may be thereby rapidly decreased to a point where it no longer destroys microörganisms.

3. *Homogeneity.*—Disinfectants should be homogeneous in composition. Substances which may be bought in pure condition or in crystalline form such as mercuric chloride, are ideal in this respect. Many of the commercial disinfectants, particularly those prepared from coal tars, may vary considerably in their composition from time to time, and consequently in their germicidal value.

4. *Solubility.*—The ideal disinfectant is one which will dissolve in all proportions in water.

5. *Nontoxic to Higher Life.*—An ideal disinfectant would be one which is nonpoisonous to man and animals. Obviously disinfectants which will kill one kind of cell and not injure another are difficult to find. Most of the valuable disinfectants are more or less injurious to tissues. Certain disinfectants, however, may be injected into the blood, exerting a more harmful influence upon microörganisms than upon tissues of the body, destroying the former without seriously injuring the latter. Such, for example, is the salvarsan used in the treatment of syphilis.

6. *Noncorrosive.*—Inasmuch as disinfectants must frequently be used in contact with metal surfaces it is highly desirable that they should not attack metals, injure fabrics, leave stains, or bleach color.

7. *Penetration.*—Disinfectants differ decidedly in their power to penetrate materials to be sterilized. An ideal disinfectant is one which penetrates rapidly and efficiently.

8. *Economy.*—The ideal disinfectant should be low in cost. Certain valuable disinfectants, because they are so expensive, can be used only in limited quantities and for special purposes. The high cost of salts of silver, for example, limit the use of silver almost entirely to medicine. If the entire water supply of a city is to be sterilized, obviously a disinfectant must be chosen which is relatively inexpensive.

9. *Power to Remove Dirt and Grease.*—This is related to power of penetration. A film of oil or grease over the surface of certain materials may wholly prevent the action of many disinfectants. Those which have power to dissolve or remove grease and other kinds of dirt are naturally more efficient.

10. *Deodorizing Power.*—A disinfectant which can combine with and destroy malodorous substances is preferable to one which does not interfere with them.

Effect of Hydrogen Ion Concentration upon Death Rates.—The addition of acid to any nonalkaline solution containing bacteria (with the consequent increase in hydrogen ion concentration) increases markedly the rate of death.

Bacteria which grow well in a relatively high hydrogen ion concentration are termed *acidophiles.* The ability of these organisms to grow in the presence of high concentrations of acid is sometimes utilized in their isolation.

Acids are most commonly used as preservatives. The most important are the *acetic* (usually in the form of vinegar) and the *lactic acids,* the latter the preservative agent in sour milk, sauerkraut, and silage.

Strong acids are those which ionize most completely. Weak acids are those which ionize poorly. Lactic and acetic acids are relatively weak acids, and must be present in relatively large amounts in order to secure a high concentration of hydrogen ions. For example, tenth normal

hydrochloric acid contains about ten times as many hydrogen ions as normal acetic acid.

In a few acids either the anion or undissociated acid molecule may also exert a disinfecting action. In some this action is much more important than that of the hydrogen ion; for example, benzoic acid is a comparatively weak acid and yet it has relatively high antiseptic or disinfecting value. This is likewise true of salicylic acid and related compounds.

Alkalinity or Alkalies.—High alkalinity, that is, high concentration of hydroxyl ions likewise exerts a destructive action upon microörganisms. *Lime* owes its value as a disinfectant to this fact. Unslaked lime (calcium oxide) in the presence of water becomes calcium hydrate and when used in strong solutions, as in whitewash, it has a marked disinfecting value. It is a valuable disinfectant to mix with stools or body excreta in order to destroy disease-producing bacteria. It should be noted, however, that upon exposure to air calcium hydrate is gradually converted into calcium carbonate which is no longer germicidal.

Some bacteria will grow in relatively alkaline solutions. For example, the organism causing Asiatic cholera, the *Vibrio cholerœ,* will grow in much higher hydroxyl ion concentration, that is, in more alkaline solutions, than will most other species of bacteria. Use is made of this fact in isolating this organism from feces, the medium being made so alkaline as to inhibit the growth of most other microörganisms rendering the securing of this organism in pure culture comparatively easy.

Salts of the Heavy Metals.—The soluble salts of the heavy metals are almost without exception more or less toxic to bacteria. In practically all cases their efficiency as disinfectants depends upon the degree of ionization. Salts of silver, mercury, and copper are of particular importance.

Silver salts, particularly *silver nitrate,* possess high germicidal power but are relatively unstable, especially in the presence of light and organic matter. Colloidal solutions of metallic silver are extensively used in medicine, the high cost of silver preventing their extensive use for other purposes.

The soluble salts of *mercury* are high in disinfecting power. This is particularly true of mercuric chloride and mercuric iodide. Mercuric chloride in solutions of one to one thousand destroys microörganisms rapidly. The soluble salts of *copper,* particularly copper sulphate, are also high in disinfecting value. It has found extensive use in the purification of water for domestic use. Copper combines very largely with the organic material present and settles out in insoluble form so that very little copper appears in the water when drawn from the tap. Within recent years, however, its use in water supplies has been very largely displaced by chlorine. Copper apparently has a particularly high power of destroying algæ, that is, it is an algicide. Solutions of copper sulphate as dilute as one part of copper to a million parts of water are effective for such purposes. Copper is the most important constituent of most *fungicides,* as Bordeaux mixture.

The Non-electrolytes.—Of the alcohols, *ethyl alcohol* (C_2H_5OH) is most commonly used. Its power of disinfection, however, is usually greatly overestimated. It is most effective in a concentration of 70 per cent. Either higher or lower concentrations will fall off rapidly in disinfecting power.

Formaldehyde (HCHO) is the most commonly used aldehyde and is most efficient as a disinfectant. It is usually sold on the market as formalin, the commercial or trade name for a 40 per cent solution of formaldehyde in water. Formalin of standard strength should not be below 37 per cent formaldehyde gas. Formaldehyde may be used

either in solution or as a gas. It finds extensive use as a fungicide in the destruction of smut spores on certain grains. For the destruction of bacteria it is most commonly used as a fumigant. The formaldehyde gas may be generated either by boiling solutions of the gas, that is, by boiling formalin, or from some of the polymers of formaldehyde, such as paraform. Formaldehyde gas is most efficient in the presence of moisture. Vessels containing formalin may be heated directly over a flame, the formalin may be sprinkled upon sheets and hung about in the room to be fumigated, or the formalin may be poured over a heap of crystals of potassium permanganate in a container that will not be corroded. In the last-named process the heat produced by the oxidation of a portion of the formaldehyde by the permanganate vaporizes the remainder.

Phenol and the *closely related compounds* are among the most satisfactory of disinfectants for general use. Phenol (C_6H_5OH) in a 5 per cent solution will destroy most non-spore-producing bacteria in a relatively short time. Some bacterial spores, however, are extremely resistant. The *cresols* or methyl phenols ($C_6H_4CH_3OH$) are the chief constituents of many of the so-called coal tar disinfectants sold on the market. The cresols are not as soluble in water as is phenol. A saturated solution contains about 2½ per cent. They are even more effective than is phenol. Frequently acids or alkalies are added to the phenols and cresols to increase their disinfecting power. *Benzoic acid* (C_6H_5COOH) and *sodium benzoate* (C_6H_3COONa), *salicylic acid* ($C_6H_4OHCOOH$), and *sodium salicylate* ($C_6H_4OHCOONa$) have been used frequently as preservatives in foods, usually in concentrations of not more than 0.2 per cent. Salicylates are generally forbidden in foods under our pure food laws, but benzoates are permitted providing the amount is fairly stated on the label. Some of the so-called canning powders sometimes used by housewives in

preserving vegetables are various mixtures of benzoates and salicylates. Their use is unnecessary and objectionable. The preservative action of the *creosotes,* the principal disinfective agency of smoke in the preservation of meat, is closely related to that of phenol.

Many of the members of the series of *aniline dyes* have decided antiseptic and disinfecting powers. They show decided differences, however, in their effect on different kinds or species of bacteria. For example, it has been found possible quite completely to inhibit the growth of most of the Gram-positive organisms by the presence of gentian violet, the Gram-negative forms growing well in its presence. The so-called Endo medium is a mixture of basic fuchsin and sulphites. It quite completely inhibits the growth of most organisms other than those belonging to the colon typhoid group of bacteria, that is to the genus *Bacterium.* Malachite green and other dyes have been used in differential media, inhibiting growth of some kinds of bacteria and not interfering with the development of others.

Oxidizing Agents.—The most important of the oxidizing agents which have been used for sterilization are oxygen in the nascent condition, chlorine, the hypochlorites, and potassium permanganate. *Ozone* (O_3) has frequently been heralded as an excellent fumigant. Various types of so-called "ozonizers" and "ozonaters" have been placed upon the market. While concentrated ozone undoubtedly has some destructive effect upon microörganisms, its presence in the air in considerable quantities is probably decidedly more injurious to human beings than it is to the microörganisms which may be present. Satisfactory and reliable methods of utilizing ozone have not been worked out.

Calcium hypochlorite and various other hypochlorites are used extensively in the purification of water. In the presence of organic matter these break up, releasing free chlor-

ine. Eventually the chlorine goes into combination with various bases and disappears in this form. The hypochlorites are therefore disinfectants which act for a short period of time only, after which their potency disappears. This makes them exceptionally valuable for certain purposes, but unreliable for others. The spore-producing bacteria are relatively resistant to sterilization with hypochlorites. Alkaline solutions of hypochlorites have also been used for wound disinfection. Such, for example, is the Dakin's solution used extensively in the sterilization of badly infected war wounds. Potassium permanganate by its direct oxidizing activity will completely destroy microörganisms in a comparatively short period of time.

Essential Oils.—The essential oils of many spices and other plants have considerable antiseptic power. Among them may be mentioned oil of cloves, oil of cinnamon, oil of mint and especially oil of eucalyptus.

Stimulation of Disinfection.—The disinfecting action of disinfectant in some cases may be materially increased by the addition of other substances. For example, the addition of alcohol to a solution of phenol materially increases the rapidity with which it destroys microörganisms. Hydrogen ions frequently increase the speed of disinfection. On the other hand disinfecting action may be materially reduced by the addition of certain substances. Sodium chloride, for example, when added to mercuric chloride, decreases ionization and correspondingly the rate at which microörganisms are destroyed.

Autogenic Products.—Substances produced by the growth of microörganisms in culture media frequently not only eventually stop their growth, but determine, in part at least, the rate at which they die off. In short, bacteria are usually eventually killed by products of their own growth. In some cases this is due to the development of acids or alkalies, but in many cases the substances produced are not

well known. Some of them are easily destroyed by heat. For example, *Bacterium coli* when grown upon a culture medium renders that medium unfit for future growth of the same organism until it has been heated. Heating apparently destroys the toxicity of the growth product.

Use in Therapeutics.—Antiseptics or disinfectants which may be used in or upon the human body are, of course, much to be desired. Such substances must have a much higher destructive action upon microörganisms than upon the tissues of the body with which they come in contact. Within the past two decades persistent effort has been made to find substances which may be injected into the body to kill certain kinds of microörganisms without injuring the body tissues. Such studies have led to the discovery of substances such as arsphenamine (salvarsan), the so-called specific for syphilis. Certain of the aniline dyes and related chlorine compounds have been found useful in wound disinfection.

In a later chapter the ability of the body to produce substances harmful to microörganisms will be discussed. It will be found that in the blood serum substances toxic to bacteria may be developed in sufficient quantities entirely to prevent their growth in the body.

CHAPTER XII

CHEMICAL CHANGES PRODUCED BY MICROÖRGANISMS

Energy Relationships

Microörganisms, like all other forms of life, as long as they are living and growing require a constant supply of energy. This is used by the organism in various ways. Energy is required in some cases for manufacture of food, in all cases for the building up of complex compounds such as protoplasm. Energy is necessary for growth and for movement. Many of the chemical changes brought about by microörganisms are the result of their methods of securing energy.

Microörganisms show great variation in the manner in which they secure their growth energy, and in the manner in which it is utilized. Some microörganisms require organic food, others are capable of manufacturing their own food. Some live under aërobic conditions, others under anaërobic conditions. A knowledge of the sources of energy available to microörganisms and of the ways in which they make use of this energy is therefore necessary.

Sources of Energy.—Apparently the common source of energy made use of by all forms of life is oxidation. An additional source of energy utilized by some forms of life is sunlight. Most species of higher plants possess the green coloring material chlorophyll which enables them to make use of the energy of the sun's rays for the purpose of manufacturing their food from inorganic compounds, primarily from carbon dioxide and water with the formation of carbohydrates, particularly starch.

Sunlight as a Source of Energy to Microörganisms.—It is somewhat doubtful whether any group of bacteria, yeasts, or molds, are capable of utilizing the energy of light for manufacture of foods from inorganic compounds. The possible exceptions are to be found in that group of bacteria termed the *Thiobacteriales* (sulphur bacteria). Certain of these species contain two coloring materials, *bacteriopurpurin*, a purple coloring substance, and *bacteriochlorin*, a green coloring substance. The organisms possessing these pigments apparently prefer to live in the presence of light and are attracted toward the more brilliantly illuminated side of the vessel in which they are growing, that is, they show positive phototaxis. That they actually make use of the sunlight, however, as a source of energy for the manufacture of foods from simple inorganic substances has not been definitely proved.

Oxidation as a Source of Energy for Microörganisms.—All bacteria, yeasts, and molds secure energy by the oxidation of compounds, either organic or inorganic. For convenience in discussion they may be divided into three groups. 1. The *prototrophic* bacteria secure energy by the oxidation of inorganic elements and compounds and utilize the energy thus secured in building up their own foods. Such organisms are totally independent of extraneous organic food substances. 2. The *metatrophic* organisms apparently require organic sources of carbon and secure growth energy usually by oxidation. They may, however, utilize inorganic compounds of nitrogen. 3. The *paratrophic* bacteria usually require both nitrogen and carbon in the form of organic compounds. For the most part they are parasitic bacteria and many of them are disease producers.

The Prototrophic Bacteria.—These are the organisms which by means of the oxidation of purely inorganic substances are able to make use of the same or other inor-

ganic substances in building up their own food. They are not the ones most commonly studied and found in the laboratory, however. Certain bacteria in the soil are known to oxidize ammonia into nitrous acid, using the energy thus secured for building up their own food and protoplasm. Still other bacteria oxidize nitrites into nitrates making similar use of the energy secured. Water in springs containing a considerable amount of hydrogen sulphide usually abound with so-called sulphur bacteria which can oxidize hydrogen sulphide to free sulphur and finally to sulphuric acid, utilizing the large amount of energy thus secured for their own growth. These sulphur bacteria also occur in the soil and are doubtless effective in oxidizing the hydrogen sulphide developed during the decomposition of organic substances. Bacteria have been discovered also which can oxidize hydrogen gas, and others which can oxidize carbon monoxide, thus utilizing them as sources of growth energy. Certain water bacteria oxidize ferrous iron to ferric iron, and manganous manganese to manganic manganese.

It is probable that in all cases organisms belonging to the prototrophic group use a considerable proportion of the energy secured in building up carbon compounds from carbon dioxide and water. The fact that they are independent of organic materials and of sunlight makes them apparently among the most primitive of living organisms.

Metatrophic Bacteria.—To this group belong the yeasts, the molds, and the most of the bacteria. These organisms require organic compounds of carbon, and by oxidation of these compounds they are able to secure growth energy. They also use these compounds in building up their protoplasm. They are in many cases able to utilize inorganic sources of nitrogen. Some forms, for example, can fix atmospheric or free gaseous nitrogen. As a result of the energy secured by the oxidation of carbohydrates many

forms among the bacteria, yeasts, and molds, can utilize nitrogen in the form of ammonia or of nitrates. Most of the bacteria producing fermentation, decay, and putrefaction belong here.

Paratrophic Bacteria.—These bacteria require particular compounds of carbon and usually of nitrogen for their development. Most of them are parasitic, living upon the tissues of plants and animals. When grown in the laboratory care must be used that the right amino acids, vitamines, and carbon compounds are supplied. This group is important because of the disease-producing organisms which belong to it.

Methods of Securing Energy by Oxidation.—It has already been emphasized that microörganisms secure growth energy by oxidation. This may occur as a result of direct oxidation of carbon or other compounds or elements by atmospheric oxygen, the oxidation of one compound and simultaneous reduction of another, and by intramolecular oxidation.

Many of the aërobic bacteria secure their growth energy by direct oxidation of inorganic or organic elements and compounds with atmospheric oxygen. As an example of this type of transformation may be cited the oxidation of ethyl alcohol into acetic acid by the vinegar bacteria of the genus *Acetobacter*. The change may be illustrated by the equation:

$$\underset{\text{Alcohol}}{C_2H_5OH} + O_2 = \underset{\text{Acetic acid}}{CH_3COOH} + H_2O$$

The same bacteria are also capable of oxidizing acetic acid to carbon dioxide and water. This may be illustrated by the following equation:

$$CH_3COOH + 2O_2 = 2CO_2 + 2H_2O$$

In these two steps the alcohol has been completely oxidized and the maximum possible amount of energy is secured from the alcohol.

Many species of bacteria, particularly the forms known as facultative anaërobes oxidize carbon compounds in the absence of atmospheric nitrogen, providing some other compound containing oxygen which may be reduced is readily available. For example, nitrates when introduced into broth enable many bacteria that would otherwise be aërobic to live under anaërobic conditions. These reduce the nitrates to nitrites, utilizing the oxygen thus secured for the oxidation of carbon compounds. It is evident that the energy yield secured by this process is not as high as by direct oxidation with atmospheric oxygen.

Many organisms are able to grow in the absence of free atmospheric oxygen because of their ability to bring about intramolecular rearrangement of atoms or intramolecular oxidation of carbon in other compounds. Certain bacteria, for example, directly or indirectly are able to transform the sugar dextrose into lactic acid. This may be represented by the equation

$$\underset{\text{Dextrose}}{C_6H_{12}O_6} = 2\ \underset{\text{Lactic acid}}{C_3H_6O_3}$$

If both dextrose and lactic acid are studied by the methods of the physical chemist it will be found that the number of calories of heat secured by the complete oxidation of one gram of dextrose is greater than that secured by the complete oxidation of the same weight of lactic acid. In other words, inasmuch as energy is indestructible, when sugar is changed into lactic acid an intramolecular oxidation has occurred with the liberation of a certain amount of energy. It is apparent that this method is least efficient of all, that is, much larger quantities of materials are needed for the development of a given amount of energy by organisms belonging to the third group than by those belonging to the first. The complete oxidation of a gram of glucose gives 3.76 large calories of heat. Similar oxidation of a gram of lactic acid will

give 3.6 large calories of heat. The energy secured by the transformation of the glucose into lactic acid is represented by the difference between these two or .16 calorie. In other words more than twenty-three times as much energy may be secured by the complete oxidation of a given amount of glucose than is secured by the changing of this amount of glucose into lactic acid.

A gram molecule of ethyl alcohol yields 325.7 large calories. A gram molecule of acetic acid yields 209.4 large calories. By the transformation of a gram molecule of ethyl alcohol into acetic acid there is released the difference, or 116.3 large calories.

Cycles of the Elements

The fact that many organisms are constantly oxidizing certain compounds and reducing others, or oxidizing compounds containing one element under certain conditions and reducing under others, makes possible the study of a cycle of changes which these elements undergo. Three of the elements are of sufficient importance from an agricultural point of view, and are sufficiently changed by microörganisms, as to warrant their discussion. These elements are nitrogen, carbon, and sulphur.

The Nitrogen Cycle.—The cycle of changes brought about by microörganisms in nitrogen compounds in nature can best be followed by reference to the diagram on page 240. Complex nitrogen compounds are constantly being broken down by a process of hydrolysis into ammonia, the ammonia oxidized to nitrates, the nitrates taken up by higher plants, converted into amino acids, and built up into proteins, thus completing the cycle. Microörganisms bringing about changes of economic significance will be discussed in much greater detail under later chapters.

Proteolysis and Ammonification.—It is perhaps most con-

venient to commence consideration of the nitrogen cycle with one of the more complex organic nitrogenous compounds. Such compounds, for example, are the proteins of plants and animals. These undergo decomposition after the death of the plant or the animal, the more or less insoluble compounds being converted into soluble forms by a process of hydrolysis. This transformation is termed *proteolysis*. Finally the nitrogen appears in the form of ammonia, so that from this point of view the full process may be termed one of *ammonification*. The first step in the process apparently is the breaking down of the proteins into the proteoses, differing somewhat in the size of the molecule though still very large but showing greater solubility. The proteins are in turn transformed into peptones, still very complex compounds. The peptones in turn are split into peptids and the peptids finally into alpha amino acids. Some eighteen or twenty different amino acids have been detected among the decomposition products of proteins. Certain species of bacteria and other microörganisms help to bring about the process termed *deaminization*, in which nitrogen is given off in form of ammonia.

A considerable proportion of the nitrogen of animal bodies is excreted in the form of urea or in some species of animals as uric acid. These compounds are likewise attacked by bacteria and are broken down into ammonia. Eventually, therefore, practically all organic nitrogenous compounds under the influence of bacterial action liberate their nitrogen in the form of ammonia. The ammonia thus produced in soil, for example, is readily available to many species of plants and to various microörganisms. Most of the ammonia, however, is rapidly converted by other bacteria by a process termed nitrification.

Nitrification.—Certain species of bacteria present in soil oxidize ammonia to nitrous acid and to nitrites. Others

convert the nitrites into nitrates. The first of these processes is termed *nitrosation*, the second *nitratation*, and the two together *nitrification*. All of these terms are misnomers. Instead of being nitrification the process is in reality an oxidation.

Denitrification.—It has been previously noted that microörganisms living in the presence of organic matter and of nitrates, in the absence of free atmospheric nitrogen, may reduce the nitrates to nitrites making use of the oxygen thus secured for oxidizing carbon compounds. Other microörganisms carry the change still farther, breaking down the nitrites with the formation of free nitrogen gas. This latter process, like the former, takes place only in the absence of free atmospheric nitrogen and is not very common in soil. However, it constitutes in a sense a leak in the nitrogen cycle, the nitrogen passing off into the free gaseous nitrogen of the air.

Nitrogen Assimilation.—The green plants as well as many microörganisms living in the soil take up nitrogen either in the form of ammonia or of nitrates, and build it up into complex organic compounds. This process is the reverse of that which we have just been considering. It is the building up of proteins from inorganic nitrogen. Animal proteins are in all cases derived directly or indirectly from plant proteins. This completes the cycle of nitrogen.

Nitrogen Fixation.—The fact that there is a leak in the nitrogen cycle, and that nitrogen may also be lost from nitrogenous compounds in the process of burning makes it evident that there must be methods whereby nitrogen can be returned from the atmosphere to the nitrogen cycle. This is in large part accomplished by certain bacteria which, as a result of the energy secured by the oxidation of carbon and other compounds, are able to take up free atmospheric nitrogen, combine it with other elements, and work it up into their own protoplasm. Such bacteria in some cases live

free in the soil. In other cases they live in the root nodules of leguminous plants.

The Carbon Cycle.—All plants and animals are constantly producing carbon dioxide during the process of metabolism. A part, at least, of the growth energy of every living cell is secured by the oxidation of carbon compounds, and carbon dioxide is eliminated. However, there are a few microörganisms and the whole group of green plants which are capable of synthesizing carbon dioxide and water into complex organic compounds, particularly carbohydrates and proteins (together with ammonia). We have constantly, therefore, in action the two processes, formation of carbon dioxide from complex organic compounds, a process of oxidation, and the formation of organic compounds from carbon dioxide, a process of reduction. Microörganisms are constantly producing considerable quantities of carbon dioxide in the decomposition of organic material. A few, however, have been noted, such as those which oxidize ammonia to nitrites, which apparently can make use of carbon dioxide in the synthesis of their own food. The carbon cycle is therefore comparatively simple, consisting of this alternate oxidation and reduction.

The Sulphur Cycle.—Hydrogen sulphide apparently originates in nature in two ways: first, as the result of the decomposition of organic compounds containing sulphur by microörganisms with the formation of free H_2S; and second, the reduction of sulphates under anaërobic conditions by microörganisms with the formation of free H_2S. Hydrogen sulphide is also found in water of certain springs.

The ordinary putrefactive and decay-producing bacteria, for the most part, are capable of liberating hydrogen sulphide from the complex protein compounds in which it is found. A much smaller number of species are capable of changing sulphates to sulphides under suitable conditions. In some cases the decomposition of sulphates to

sulphides may give rise to such large amounts of hydrogen sulphide as to constitute a nuisance. This is one of the difficulties found, for example, in the operation of certain types of septic tanks in sewage disposal. Where cities have a water supply which is unusually high in sulphates, its mixture with organic material and retention under anaërobic conditions is apt to lead to the formation of undesirable amounts of hydrogen sulphide.

Certain bacteria already noted, particularly forms belonging to the group of so-called sulphur bacteria, are able to oxidize hydrogen sulphide to free sulphur, and free sulphur to sulphuric acid or to sulphates.

The sulphur cycle is completed by the assimilation of sulphates by higher plants and the building up of the sulphur into more complex organic compounds. As with the nitrogen and the carbon cycles we have constant changes, oxidation occurring in the presence of free atmospheric oxygen, reduction in the absence of atmospheric oxygen and assimilation by growing plants. Some authors, particularly in soil bacteriology, have used the term *sulphofication* in a sense analogous to nitrification, and *desulphofication* for the reduction process, analogous to denitrification.

CHAPTER XIII

MECHANISM OF CHEMICAL CHANGES PRODUCED BY MICROÖRGANISMS—ENZYMES AND FERMENTATION

THE bacteria, yeasts and molds must secure all of the materials useful to the cell by a process of absorption or of osmosis. All organisms grow only in contact with water. The substances to be used must be dissolved in the water outside the cell and diffuse through to the interior. There is nothing corresponding to a mouth through which solid particles may enter. It is apparent, therefore, that microörganisms must bring about changes in substances outside the cells in order either that they be made soluble in water, thus capable of diffusing into the cell, or so changed that even though soluble they may diffuse into the cell and be utilized. Further, the cell must make use of materials which diffuse to the interior in various ways. Sometimes these must be broken down into simpler compounds. Usually they must be oxidized directly or indirectly and the energy thus secured utilized for growth purposes. Certain of the compounds introduced in some instances may be reduced. Carbon dioxide and water, for example, diffusing into the cell may be combined to form carbohydrates.

The fact that microörganisms can bring about changes in the chemical nature of compounds led many years ago to their designation as *organized ferments.* They were contrasted with so-called body secretions such as the gastric juice, which could also bring about fermentative changes and digestion, and which were termed *unorganized ferments.* Later it was discovered that many microörganisms excreted substances which brought about changes outside the cell

body in just the same manner that the juices secreted by the glands of the stomach act outside the stomach walls, that is, away from the cells which produce these substances. In other words, it soon became evident that the so-called organized ferments acted at least in part through the agency of unorganized ferments which were excreted. However, it was not found possible to find unorganized ferments excreted from the cell which would bring about all of the chemical changes produced by microörganisms. Finally it was discovered that certain of these substances capable of bringing about chemical changes were constantly retained inside the cell and usually worked there. For example, it was found that yeast was incapable of producing alcohol and carbon dioxide from sugar outside the yeast cell; however, by suitable methods it was found possible to press out the yeast contents, filter them entirely free from living cells, and demonstrate that this material could bring about alcoholic fermentation. It was shown, in other words, that the changes brought about inside the cell are brought about by agencies similar to those which may be excreted. Finally the term *enzyme* was introduced to indicate any substance produced by living cells capable of bringing about chemical change. Instead of classifying ferments into organized and unorganized, differentiation was made between enzymes that act outside the cell, that is, that are *extracellular*, and those that act inside the cell, that is, that are *intracellular*. It is quite possible that all of the chemical changes observed produced by microörganisms are the direct or indirect result of enzyme action. It is, therefore, important that the nature of the enzymes produced and the reactions which they bring about should be understood.

Enzymes

Many substances are known in nature which can bring about chemical changes in other compounds without becom-

ing themselves a part of the final product, or without being used up in the process of being used. For example, a platinum sponge thrust into a jet of hydrogen will become hot, finally glow and ignite the hydrogen. It has the capacity of adsorbing upon its surfaces the hydrogen and oxygen, and these are brought into such intimate contact that they unite to form water, giving off heat. The platinum apparently does not take part in the chemical change; certainly it does not become a part of the final product, water. Substances which can act in this fashion, that is, can bring about chemical changes without themselves becoming part of the final product and that are not used up by being used, are produced by many cells, probably by all cells. Such substances are termed *enzymes*. Enzymes, therefore, may be somewhat inadequately defined as organic catalysts.

Enzymes are undoubtedly extremely complex organic compounds. They are relatively unstable, that is, they are easily destroyed by unfavorable conditions. High temperatures, excessive acidity or alkalinity, the presence of certain chemicals, and light, all tend to destroy them. There is, therefore, in any enzyme solution an inevitable deterioration.

It will be noted that the main function of enzymes is apparently to bring about chemical changes. Methods of isolating and identifying the enzymes themselves have not proved successful in most cases at least. The exact chemical composition of none of the enzymes is as yet known. In fact there seems to be no chemical test which will enable one to recognize the presence of enzymes other than that of allowing the enzyme to act under suitable conditions, and noting the changes brought about, the amount of enzyme present being deduced from the amount of change produced.

The action brought about by an enzyme is probably in general *reversible*; that is, an enzyme brings about a change

in a compound until the new compound formed is in equilibrium with the unchanged compound. In other words, enzymes not only can tear down, they are not only analytic, but they are synthetic as well, being capable of building up complex compounds from the simpler. For example, the enzyme maltase, when brought in contact with maltose in solution in water, changes most of this maltose into glucose. Finally an equilibrium is established, there being present a small amount of maltose and a large proportion of glucose. Conversely when maltase is placed in a solution containing glucose it will build up a small amount of maltose (isomaltose) until again the solutions are in equilibrium. Undoubtedly in the body and in the cells of plants and animals this synthetic action is quite as important as the analytic.

For convenience in study the enzymes may be divided into three principal groups. The first includes those whose function, from the standpoint of the cell, is primarily *digestive.* These enzymes, for the most part at least, act by a process of hydrolysis, that is, one or more molecules of water are incorporated into the molecule which is to be changed, which then breaks down into two or more simpler molecules. Most of the extracellular enzymes are of this nature. They are collectively known as *hydrolases.* The second group includes those enzymes that bring about changes primarily *oxidative* in nature. The changes which they bring about usually yield energy to the cell. They may, in a sense, be termed the respiratory enzymes. In some cases they function by the addition of atmospheric oxygen or oxygen derived from other compounds easily reduced to the compound, usually a carbon compound, to be changed. Such enzymes are generally termed the *oxidases.* Others bring about a rearrangement of atoms within the molecule, an intramolecular oxidation, and the molecule splits into two or more new molecules. Such enzymes are frequently

termed the *splitting enzymes* or *zymases.* Third, still other enzymes are primarily concerned in reduction processes. It is evident that wherever food material is built up from simple inorganic substances that there must be reduction taking place. The building up of sugar, for example, from carbon dioxide and water implies the reduction of carbonic acid (H_2CO_3) to formaldehyde (HCHO), and the polymerization of this product into sugar. Such enzymes are *reductases.*

The hydrolytic enzymes are both extracellular and intracellular. Most of the oxidizing, splitting, and reducing enzymes are intracellular.

From the standpoint of agricultural or economic significance, the hydrolytic and oxidizing enzymes are by far the most important. The hydrolytic and oxidative enzymes produced by microörganisms will therefore be discussed.

The Hydrolytic Enzymes or Hydrolases.—The hydrolases, that is, the enzymes which bring about splitting of organic compounds by the addition of water, are for convenience divided into three groups, the carbohydrases, the proteases or amidases, and the esterases or lipases. The first group, the *carbohydrases,* bring about hydrolytic decomposition of carbohydrates and related compounds. The *proteases* hydrolyze the proteins and their various decomposition products to amino acids. The *esterases* or *lipases* hydrolyze the esters and particularly the glycerine esters or fats. Enzymes belonging to all of the groups are known among microörganisms.

Carbohydrases.—Enzymes capable of hydrolyzing the various complex carbohydrates down to the simple sugars are known. The principal sub-groups of carbohydrases are the *cytases* and *cellulases* which attack the celluloses and hemicelluloses, the *pectinases* attacking pectin, the *diastases* and *inulases* attacking starch and inulin, and the *invertases,* which hydrolyze the disaccharides to simple sugars.

The celluloses, hemicelluloses and vegetable gums are carbohydrates having very large molecules made up by the polymerization of the simple sugar radicles. Inasmuch as cellulose and hemicellulose and closely related compounds constitute a large proportion of the bodies of the higher plants, it is evident that were it not for the decomposition brought about by microörganisms, there would be large accumulations of such materials in soil. Many species of molds and bacteria are known which can decompose cellulose, breaking it down into simpler compounds. The enzymes capable of bringing about these changes are usually termed *cytases* or *cellulases*. A few organisms are also known which can decompose vegetable gums which are closely related chemically to the hemicelluloses. The development of cytase by microörganisms may be demonstrated by mixing finely pulverized cellulose or hemicellulose with suitable melted agar, and pouring into a Petri dish. Bacteria or molds capable of digesting this carbohydrate will be visible because of the clear ring surrounding the colony. By the action of the cytase undoubtedly considerable quantities of carbohydrate food are released to other soil bacteria, not capable of attacking the cellulose directly.

Pectinases are enzymes capable of dissolving or digesting the carbohydratelike substance called pectin. This is present in most plants, constituting the layer between the cellulose walls of adjoining cells. In morphologic botany it is called the middle lamella. It may be termed the glue which holds plant cells together. Certain microörganisms, particularly among the molds and the bacteria, secrete pectinase which dissolves this middle lamella and frees the cells from each other. From an agricultural point of view this is particularly important in the retting of flax and hemp, in which molds or bacteria dissolve out the pectins which bind the linen fibers and hemp fibers together; it is important also with certain of the disease-producing bacteria, such as

the organism causing cabbage black rot, which softens the middle lamella over large areas of the leaf causing the cells eventually to die and decompose.

Among the higher plants the most common reserve carbohydrate materials stored up are starch and inulin. Starch is a polymer of dextrose, inulin a polymer of levulose. Starch is attacked or hydrolyzed by the enzyme *amylase (diastase)* which changes it into dextrin and finally into the sugar maltose ($C_{12}H_{22}O_{11}$). This enzyme is produced by sprouting seeds, is present in most leaves and growing tissues of plants, and is formed by many species of bacteria and molds, though, so far as is known, not by any of the yeasts. The property of amylase production or starch digestion may be demonstrated with molds or bacteria by the use of starch plates, that is, by plates poured from agar containing dissolved or suspended starch. Those organisms which digest the starch will change its appearance from milky or opaque to transparent and clear. The ability to saccharify or liquefy starch possessed by certain molds has been made use of in industries to prepare starchy materials for alcoholic fermentation by yeasts. These will be discussed later. It should be noted that not all organisms which attack starch and break it down into simple compounds, change it into the intermediate simple sugars. For example, the *Bacterium aërogenes,* although it is able to produce gas and acid in starch solutions, does not develop the enzyme amylase.

The carbohydrate inulin is very common as a reserve food product in the plants belonging to the sunflower or composite family. The sunflower plant itself, chicory, dandelion and dahlia all contain considerable amounts of this carbohydrate. The enzyme *inulase* converts (by hydrolysis) inulin into levulose. Certain molds are known which produce inulase. Whether or not this is also produced by bacteria has not been adequately demonstrated. There are

bacteria known which will ferment inulin but whether the inulin is attacked directly or first broken down into levulose has not been proved.

Three of the common disaccharides are frequently used in the study of microörganisms. These are sucrose or cane sugar, maltose or malt sugar, and lactose or milk sugar. These are hydrolyzed by the enzymes *sucrase, maltase* and *lactase* respectively. The reaction may be indicated as follows:

$C_{12}H_{22}O_{11}$	$+ H_2O +$	invertase $=$	$C_6H_{12}O_6$	$+ C_6H_{12}O_6 +$	invertase
sucrose		sucrase	dextrose	levulose	sucrase
or		or		or	or
maltose		maltase	dextrose	dextrose	maltase
or		or		or	or
lactose		lactase	dextrose	galactose	lactase

Many species of molds and yeasts are known which produce one or more of these enzymes. Common bread yeast, for example, produces the enzymes sucrase and maltase but not lactase. Certain of the lactic yeasts, however, are able to produce lactase as well. These enzymes are collectively known as *invertases,* and the process of hydrolysis of a disaccharide is termed an inversion. It should here be remarked as under starch fermentation, that many bacteria are capable of attacking disaccharides without hydrolyzing them into the simple sugars; that is, the fact that a microorganism can ferment sucrose does not indicate that it necessarily produces the enzyme sucrase, or that dextrose and levulose are intermediate products in the fermentation.

Proteases and Amidases.—In the discussion of the nitrogen cycle it was noted that proteins are exceedingly complex nitrogenous organic compounds built up by the polymerization of alpha amino acids. The hydrolysis of these compounds is brought about by a group of enzymes termed proteases and amidases. Those most common are the *pepsins,* the *trypsins,* and the *erepsins.* The pepsins are enzymes

capable of attacking proteins, breaking them down into peptones, and perhaps in some cases into polypeptids and peptids. They require an acid medium. Trypsins are enzymes capable of attacking proteins, breaking them down through the stage of peptones into the peptids, the amino acids, and some cases even to ammonia. They require an alkaline medium. The erepsins resemble trypsins in their end products, but attack only compounds somewhat less complex than the proteins. They will attack, for example, the proteoses and the peptones. The general types of reaction brought about may be illustrated by the hydrolysis of a dipeptid into amino acid.

$$\begin{array}{l} R\cdot CH\cdot COOH \\ \quad\cdot \\ \quad NH \\ \quad\cdot \\ R\cdot CH\cdot CO \\ \quad\cdot \\ \quad NH_2 \end{array} + H_2O = \begin{array}{l} R\cdot CH\cdot COOH \\ \quad\cdot \\ \quad NH_2 \\ \quad\cdot \\ R\cdot CH\cdot COOH \\ \quad\cdot \\ \quad NH_2 \end{array}$$

The amino acids may be regarded as the building stones out of which proteins are constructed. The cement which binds them together is the abstraction of water. Enzymes which attack proteins insert water and the compounds fall apart on the lines of cleavage.

Microörganisms are known to produce various types of proteases. One of these most commonly observed in the laboratory is gelatinase, the enzyme capable of liquefying gelatin. Bacteria have been described which will liquefy egg white and digest blood serum. These changes are sometimes noted as cultural characteristics of microörganisms studied. Many microörganisms, too, are able to digest the casein of milk in which they are grown, either with or without the preliminary coagulating action of the so-called lab enzyme or rennet.

Esterases and Lipases.—Fats, that is, the glycerine esters of the fatty acids, are hydrolyzed by lipases into the corre-

sponding fatty acids and glycerine. For example, certain microörganisms live in butter and produce lipases which may decompose stearin in accordance with the following reaction:

$$\underset{\text{Stearin}}{(C_{17}H_{35}CO_2)_3C_3H_5} + 3H_2O + \text{lipase} = \underset{\text{Stearic acid}}{3C_{17}H_{35}COOH} + \underset{\text{Glycerine}}{C_3H_5(OH)_3} + \text{lipase}$$

Lipases have been described for certain species of molds but they are not very common among the bacteria. They may be demonstrated by preparing an emulsion of a suitable fat, such as butter fat, or one of the vegetable oils, in agar. Microörganisms grown upon the surface of such a medium will show ability to hydrolyze the fat by the clearing of the medium immediately surrounding the colony.

The Splitting and Oxidizing Enzymes.—The most important of the so-called splitting enzymes are the *zymase* of yeast, and the *lactacidase* of the lactic acid bacteria. The enzyme zymase in the presence of phosphoric acid is capable of transforming the monosaccharide sugars such as dextrose, into alcohol and carbon dioxide. There probably are one or more intermediate steps in the process, but the transformation may be illustrated by the equation:

$$\underset{\text{Dextrose}}{C_6H_{12}O_6} = \underset{\text{Alcohol}}{2C_2H_5OH} + 2CO_2$$

This enzyme is characteristic of most species of yeasts; it is present also in a few of the molds and probably in some species of bacteria. The enzyme is intracellular. Lactacidase likewise transforms sugars, including the monosaccharides, with the end product lactic acid. Here again there are probably several intermediate steps in the process, but the ultimate transformation may be indicated by the equation:

$$\underset{\text{Dextrose}}{C_6H_{12}O_6} = \underset{\text{Lactic acid}}{2CH_3CHOHCOOH}$$

This reaction is brought about within the cells of certain species of bacteria, and probably to a less degree by certain species of yeasts and molds. The enzyme lactacidase, however, has apparently never been secured outside the bacterial cell.

Oxidizing enzymes which combine organic compounds with oxygen from the air are comparatively common in microörganisms. Probably most of them are intracellular. But little detailed work has been done upon them. Much more is known concerning the oxidases developed by higher forms of life, particularly the higher plants. One of the most striking of the oxidative changes is that produced by members of the genus *Acetobacter* in the oxidation of alcohol to acetic acid. This may be illustrated by the following equation:

$$\underset{\text{Alcohol}}{C_2H_5OH} + O_2 = \underset{\text{Acetic acid}}{CH_3COOH} + H_2O$$

Many color reactions are the result of the presence of oxidases. This is particularly true among higher forms. The bruised surfaces of many fruits, for example, are known to change color because of the action of oxidases in the cells which are exposed to the air. It is quite possible, though by no means demonstrated, that the oxidizing activity of organisms belonging to the genera *Nitrosomonas* and *Nitrobacter* is due to the presence of enzymes of this group. Probably all cells contain oxidizing enzymes capable of releasing energy from the food, particularly from carbohydrate substances.

Fermentation

Fermentation may be very broadly defined as any chemical change brought about directly by microörganisms or indirectly as the result of the action of enzymes or other substances produced by them. It is sometimes somewhat more narrowly defined to include only chemical changes

brought about in carbohydrates. The term *putrefaction* is sometimes applied to decomposition, particularly of protein materials, occurring under more or less anaërobic conditions, usually with the development of malodorous substances. *Decay* is similar decomposition occurring usually under aërobic conditions without noteworthy development of malodorous compounds.

Practically all fermentation, putrefaction, and decay in nature occur as the result of the activity of microörganisms, that is, of the bacteria, the yeasts and the molds.

The Origin of the Products of Fermentation.—The products developed during the process of fermentation may be grouped under four headings, using the origin as a basis for the classification.

1. *The Analytic Action of Extracellular Enzymes.*—The digestive changes brought about by extracellular enzymes break down compounds on the exterior of the cell into simpler compounds. These are usually formed greatly in excess of the immediate needs of the microörganisms. Such, for example, is the transformation of starch into maltose by certain molds, or the digestion of casein or gelatin by bacteria.

2. *The Analytic Action of Intracellular Enzymes.*—Many of the intracellular enzymes are active in bringing about changes in those food substances which diffuse into the cell. Such, for example, is the origin of alcohol and carbon dioxide in yeasts, and of lactic acid in the lactic acid bacterium. It may be noted furthermore that the activity of the analytic enzymes contained within a cell do not cease necessarily with the death of that cell. Such enzymes as continue to act after the death of the cell and bring about more or less decomposition of the cell substance itself are termed *autolytic.* In an old culture in broth in which most of the organisms have died, the cells will be found more or less autolyzed; and chemical test will reveal substances

present in the medium which have dissolved out from the bacterial cell and which are not present in rapidly growing young cultures. Sometimes these products resulting from autolysis or self-digestion are poisonous in nature.

3. *The Synthetic Action of Enzymes.*—It will be recalled that not only are enzymes analytic in their action, but they are synthetic as well. Many times complex compounds are produced from simple compounds as a result of bacterial activity. Such, for example, are the gums and the slimes of various species of bacteria. The organism producing ropy milk, for example, polymerizes the sugars present into much more complex gums, usually galactans. The toxins and the enzymes themselves are likewise the result of synthetic action.

4. *Secondary Action of Growth Products.*—Some of the ultimate products of the action of microörganisms are secondary, that is, products formed within a cell may upon excretion bring about changes in other substances wholly outside of the cell. For example, certain bacteria growing in milk containing calcium carbonate would produce lactic acid, this, upon diffusing from the cell, would form calcium lactate, with the evolution of carbon dioxide. It can scarcely be said that the carbon dioxide is developed as a result of the direct action of the microörganism, but that it is the result of a secondary change. Some of these secondary reactions are of considerable importance in the soil. The insoluble phosphates, for example, are in part at least made soluble and available to the plant roots through the action of secondary products of the growth of bacteria. Not infrequently in fermenting materials gaseous hydrogen is developed. This as nascent hydrogen may exert a powerful reducing action upon various substances present. For example, levulose when fermented by certain bacteria is transformed in part into mannitol, that is, the sugar is changed into the corresponding polyatomic alcohol.

SECTION IV

BACTERIA IN TECHNICAL AGRICULTURE AND THE INDUSTRIES

CHAPTER XIV

RELATIONSHIPS OF MICROÖRGANISMS TO THE PRESERVATION OF FOOD

Agencies which Bring about Deterioration of Foods.—The agencies which bring about deterioration in foods and food products may be grouped under two general headings: the *intrinsic*, including those which are normally present in the food itself and have not been derived from outside influences; and second, those which may be termed *extrinsic*, that is, those which enter the food at some time during the process of preparation or ripening. To the first group belong the so-called autolytic enzymes. In certain fatty foods, for example, there may be hydrolyzing enzymes which will bring about rancidity. In fruits and certain vegetables the enzymes which normally bring about ripening may bring about a condition of overripeness. Certain of the oxidizing enzymes, such as those present in fruits, may produce discoloration. Of the extrinsic agencies capable of bringing about food deterioration, there are the bacteria, the yeasts and the molds together with the enzymes which they produce.

In general the bacteria thrive best in nitrogenous foods containing considerable proportion of water and not too much acid; they grow best in a medium which is nearly neutral. The yeasts, for the most part, grow best in a medium rich in carbohydrates, particularly in the fermentable sugars, and preferably in the presence of some acid. Like the bacteria, they prefer a considerable amount of moisture. The molds will grow in media somewhat drier and of almost any type. Certain molds will grow on foods

that are slightly acid as well as upon those which are neutral or slightly alkaline.

Methods of Preventing Deterioration.—In order to prevent deterioration food must be kept so that there can be no appreciable activity of autolytic enzymes and no growth of bacteria, yeasts, or molds. This may be accomplished by heating to a temperature and for a time sufficient to destroy all microörganisms and enzymes present, or by the process termed pasteurization, whereby those organisms capable of bringing about detrimental changes are destroyed without necessarily making the material sterile; by holding the foods at temperatures so low that undesirable changes cannot be brought about; by the presence of chemicals which will act as preservatives or antiseptics; and finally by the elimination of water to such a degree that microörganisms cannot grow and enzymes cannot act.

Sterilization by Heat.—If food materials are heated to such a temperature and for such a time as will destroy all of the living microörganisms present, and so sealed that microörganisms from the environment cannot come in contact, they may be preserved indefinitely. The process most used for this purpose is that commonly termed canning, in which the food material either before or after sterilization is hermetically sealed in containers. Meat, fruits, vegetables, and in general those foods not seriously damaged by heat and which cannot readily be preserved by drying, are utilized.

In any method of canning it is necessary that the food be properly prepared, placed in suitable containers, the air exhausted and the food hermetically sealed, and sterilized. We are here concerned primarily with the factors which determine the time and the temperature necessary for sterilization.

The first of the factors determining the time necessary for sterilization is the *initial infection,* its extent, and the kind.

It has been previously learned that bacteria are not killed off instantly by heat, but that the temperature determines the rate of death or rate at which the microörganisms die off. Other things being equal, the larger the initial number of microörganisms to be destroyed the longer will be the time necessary for their destruction. It is also apparent that the kinds of organisms present may have some influence. The spore-producing bacteria are more difficult to destroy than the nonsporulating types. A process commonly used preliminary to canning is termed *blanching*. The food products, usually vegetables or fruits, are dipped first into hot water, then into cold water. This process shrinks the tissues somewhat, makes them somewhat firmer, and tends to set the color, but also washes off many of the bacteria which were present, thus reducing the initial infection and possibly thereby somewhat decreasing the time necessary for sterilization. It is sometimes stated, furthermore, that bacteria are more easily destroyed when first heated, then cooled (or chilled) and reheated. Several tests on this point have failed to show any marked effect of this "cold shock."

The second factor in determining time of sterilization is the size of the food container or can. It is evident that the center of any can is that portion which last reaches the desired temperature. The larger the container the longer will it take this central portion to assume the desired temperature.

The third factor is the ease with which heat may penetrate to the center of the canned material. Where the particles of food are solid and are surrounded by water or thin syrup, convection currents are at once set up in the water and the interior heats relatively quickly by a process analogous to that used in a hot water system in the heating of a home. If the material lying between the food particles is viscous or gelatinous, or there is not much, if any, free

water, convection does not occur, and heat passes to the center much more slowly and only by conduction. Cans of corn heat more slowly at the center than cherries. Substances like pumpkin paste and spinach heat most slowly of all. With the latter the rate of heat conduction is practically the same as the rate of heat conduction through water.

Fourth, the hydrogen ion concentration, that is, the actual acidity of the food material, is also important. It is common experience that foods such as peaches or apples are much more easily sterilized by heat than vegetables such as peas, beans and corn. The presence of acid greatly increases the death rate of microörganisms at high temperatures.

Fifth, in some commercial canneries devices (agitators) are employed to keep the canned foods continually in motion during sterilization. This induces constant mixing of the food material and increases markedly the rate at which the heating will occur. Agitation acts in very much the same fashion as convection currents in water. The method of cooling and the time required will also determine to some extent the efficiency of sterilization.

Sixth, the temperature which must be employed is of importance. Home canning is ordinarily accomplished by the use of boiling water, and 100° c. is the highest temperature available. By the use of pressure cookers or in commercial establishments by the use of large autoclaves or pressure cookers, higher temperatures may be employed and the length of time required materially decreased.

Several methods having their counterpart in commercial processes have been utilized in the home canning of foods, important among them being the *intermitten process* of sterilization and the *cold pack process.* In intermittent sterilization it is customary to heat the food to be preserved in its container for a certain length of time, frequently an hour, on each of two, three or more successive days. The

theory is that the first heating will destroy all except the spores of the bacteria, these will germinate during the next twenty-four hours and be destroyed as vegetative cells upon the next heating or quite certainly upon the third. In the cold pack process the food material is heated in boiling water or flowing steam at approximately 100° C. for such time as will make certain the destruction of all microörganisms, or at least those which might bring about deterioration.

Not all canned foods, even though they may keep perfectly well, are completely sterilized. It has been determined as the result of many studies within the last few years that the microörganisms which may bring about deterioration in canned foods are: first, those which may enter the food as the result of defective sealing; second, those aërobic spore-producing bacteria which normally are unable to grow in canned foods because of the complete exclusion of air and which may find conditions suitable for growth if air is admitted due to defective sealing; third, anaërobic spore-producing bacteria capable of growing at normal temperature occasionally escape sterilization and produce deleterious changes, frequently accompanied by evolution of gas and the development of malodorous and bad tasting compounds, and sometimes poisons or toxins (such as *Clostridium botulinum*); and lastly, certain of the most resistant of the spore-forming bacteria belonging to the group of thermophiles. These spores of thermophilic bacteria will not germinate unless the canned food is held at a high temperature for some time. When canned foods are not adequately cooled in a commercial cannery and are stacked away in a warehouse, they may retain their heat for days or even weeks and conditions be particularly good for the development of the thermophiles. The same may happen when canned goods are stored in hot climates.

Food Preservation by Pasteurization.—Pasteurization is that process of food preservation in which the food is heated to a temperature sufficient to destroy certain types of undesirable bacteria, but not necessarily to destroy all living microörganisms present. The process was first used with wines and beers in which it was desirable to destroy certain nonspore-producing bacteria. Heating such materials to high temperatures would injuriously affect their flavor. By careful tests the time and temperature sufficient to destroy the undesirable organisms may be determined without injuring the flavor. The process has in recent times come to be used most extensively with milk. In the modern milk pasteurizing plants, however, the effort is made to heat the milk to that temperature and for a sufficient length of time to kill all disease-producing germs. Other kinds of organisms may not be destroyed. Milk, or more particularly cream, may also be pasteurized to destroy microörganisms which may give undesirable flavors or aroma to the cream products, particularly butter. It is evident that pasteurization of milk is primarily a sanitary measure rather than a means for increasing its keeping qualities.

Preservation by Low Temperatures.—The rate at which microörganisms grow and at which enzymes act decreases with decrease in temperature. Articles of food which may be frozen without damage may be preserved almost indefinitely, although even in frozen foods, such as poultry and fish, there is a slow deterioration, probably due to the action of enzymes. Other foods may be kept at low temperatures above the freezing point. Such, for example, are eggs and various other foods. It should be noted that at low temperatures the types of organisms which may grow may differ from those which develop at higher temperatures and the changes brought about will not be the same.

Preservation by Chemicals.—Some foods naturally contain substances which are preservative, for example, many

of the spices contain essential oils in sufficient quantities to have a decided preservative action. It is a well-known fact in the household that certain acid fruits and vegetables, such as stalks of rhubarb and the fruit of cranberries and gooseberries, may be preserved for considerable lengths of time in water without any sterilization.

Another class of foods is preserved as a result of chemicals produced during the process of fermentation. Without exception these are foods relatively high in sugar. Sauerkraut, for example, is preserved as the result of development of lactic and some acetic acid by bacterial growth. As long as it is kept from the air, deterioration will not take place after the acid has been developed. Dill pickles are prepared in a somewhat similar fashion. The process of preparation of silage from corn constitutes another example.

In some cases chemicals are added directly to foods in order to preserve them. The preservative may be some organic food acid such as acetic. Vinegar is used for pickling meats, fruits, and vegetables. Sodium benzoate in small quantities, although not advocated, is nevertheless permitted by our pure food laws, providing the quantities added are plainly indicated. Still other chemicals are used, such as spices and the essential oils. Certain preservatives are in general forbidden by our pure food laws. Such, for example, are salicylic acid and the salicylates and formaldehyde.

Preservation of Foods by Drying.—The amount of water which must be removed from any food material in order to prevent the growth of microörganisms will depend upon several factors, most important being the amount of soluble materials present in the food, the kinds of solutes present, the distribution of the water, the kinds of organisms or enzymes present, and the method of desiccation.

The amount of material in solution in the water in a food

determines osmotic pressure, and this is an important factor in determining the ability of microörganisms to grow. Most microörganisms cannot develop in concentrated sugar solutions. Because of their relatively high sugar content, many fruits are in consequence readily preserved by drying, and may contain a much higher percentage of water than could other carbohydrate foods, such as flour, without deterioration.

The amount of moisture that must be removed may also in part be determined by the concentration of acids present. Essential oils are also important in some instances, although the drying process may occasionally remove some of these. The malic acid of the apple is a factor of no inconsiderable importance in preserving dried apples.

The amount of water available for the growth of microorganisms is not always the amount which is revealed upon comparison of the moist and dry weight of the food material. Many foods hold their water tenaciously, not yielding it readily to microörganisms. Then, too, in some instances the water is not equally distributed throughout the food material. Butter, for example, retains water in the form of small droplets. Butter, therefore, containing 15 per cent of moisture may afford much better moisture conditions for microörganisms' growth and yield up moisture more readily than some other food containing 30 per cent. When the butter fat is completely freed from water it does not change readily. During the process of drying, furthermore, external layers of the food may be changed so as to render the penetration of microörganisms difficult. For example, meats which are dried or partially dried soon come to have the external layers saturated with fats and oils, constituting a waterproof exterior through which microörganisms cannot readily penetrate.

The kinds of organisms present or the nature of the enzymes may also determine the amount of moisture which

must be removed in order to make food keep. In general bacteria seem to require somewhat more available moisture than do the molds. Yeasts and molds grow fairly well in solutions that are somewhat acid, the yeasts requiring also sugar for their development.

The methods used in the process of desiccation also influence the keeping qualities. Certain fruits, for example, are generally sulphured, that is, exposed to the fumes of SO_2 or sulphurous acid in order to bleach them. This effectually destroys many microörganisms which are present. Other foods may be exposed to the direct rays of the sun during the process of drying and the microörganisms present destroyed.

Many methods are used for the drying of foods. Drying is expedited by the use of high temperatures, by the use of air which is relatively dry, by increasing the rapidity of air circulation by blowing or fanning, and by the use of a partial vacuum.

Dried foods may be divided into groups, using as a basis of classification the proportion of carbohydrates, fats, or proteins, present.

The carbohydrate foods such as grains, and the flours and meals produced from them usually are sufficiently dried during the process of ripening and manufacture so that they will keep indefinitely. Fats and oils usually keep well providing they are completely freed from water. Proteins are usually somewhat more difficult to dry and to preserve.

Many starchy products produced by the baker and the manufacturer, such as macaroni, vermicelli, yeast cake, crackers, biscuits, etc., are dried for preservation. Methods have also been developed for the rapid desiccation of vegetables and fruits.

Many dried fruits may contain as much as 30 per cent of moisture and yet not spoil. Syrups, molasses, sorghum, jams, jellies, preserves, etc., are readily preserved because

of the concentration of sugar present. Milk powder is prepared by several processes, among them the forcing of milk in the form of fine spray into a heated compartment from which the air has been partially exhausted. As it falls to the bottom it is transformed into a fine, dry powder which keeps well. Jerked meat, smoked meat, fish, and beef extract are preserved by the removal of water. Eggs are sometimes dried, and find extensive use among bakers.

CHAPTER XV

FERMENTATION OF CARBOHYDRATES—ALCOHOLIC FERMENTATION IN FOODS—COMMERCIAL PRODUCTION OF ETHYL ALCHOL—FUSEL OILS

One of the commonest and most important of the changes brought about in carbohydrates by microörganisms is that known as alcoholic fermentation, that is, the transformation of monosaccharides into ethyl alcohol and carbon dioxide. This type of fermentation is important in the preparation of certain beverages, in the manufacture of distilled liquors, and particularly in the preparation of commercial alcohol, bread and other foods. Inasmuch as yeasts are the most active of the organisms in bringing about alcoholic fermentation, consideration of the preparation of commercial yeasts is of importance.

Agencies Active in Alcoholic Fermentation.—A few bacteria are known which may produce alcohol in the process of fermentation. The amounts formed, however, are small, both actually and relatively to the amounts of other fermentative products. Certain species of molds, particularly when grown in sugar solutions under anaërobic conditions, can produce somewhat larger amounts of alcohol. From a practical point of view, however, all alcoholic fermentation of importance is brought about by members of the yeast genus *Saccharomyces*.

The transformation of sugar into alcohol and carbon dioxide may be represented by the equation:

$$\underset{\text{Dextrose}}{C_6H_{12}O_6} = \underset{\text{Ethyl alcohol}}{2C_2H_5OH} + 2CO_2$$

The intracellular enzyme zymase has been found capable

of bringing about this change. It may be prepared from yeast cells by grinding them with fine sand and diatomaceous earth until the cell bodies have been ruptured, then squeezing them by means of a hydraulic press to force out the cell juices. Under suitable conditions these cell juices containing zymase will produce transformation of sugar into alcohol and carbon dioxide. Apparently zymase will not act except in the presence of phosphates or phosphoric acid. Probably there is first brought about a union of the hexose sugar and the phosphoric acid to form hexose phosphate, and this is then decomposed by the zymase with the formation of alcohol and carbon dioxide.

Preliminary Preparation of Carbohydrates for Yeast Fermentation.—It has already been emphasized that yeasts are able to act directly upon monosaccharide sugars only with the production of carbon dioxide and alcohol. Carbohydrates in nature frequently exist in more complex forms, as disaccharides and polysaccharides. These must first be transformed into the monosaccharides before they can be fermented by the yeast.

The extracellular digesting or hydrolyzing powers of yeasts show marked differences. None are known which are able to attack the complex polysaccharides, such as starches and cellulose. Most species, however, are able to produce the enzyme maltase which hydrolyzes maltose to two molecules of dextrose. Likewise most species produce the enzyme sucrase which hydrolyzes sucrose or cane sugar into dextrose and levulose, and a few species are also known which produce the enzyme lactase, hydrolyzing lactose or milk sugar into dextrose and galactose. It is apparent, therefore, that no preliminary treatment is necessary for the fermentation of cane sugar or malt sugar by yeast, and that some yeasts can also ferment lactose or milk sugar.

In manufacture of alcohol generally the polysaccharides, particularly starch and cellulose, constitute the cheapest

and most easily available form of carbohydrate. Inasmuch as these cannot be attacked directly by yeasts, if they are to be used in alcoholic fermentation they must be hydrolyzed into sugars which can be directly attacked. Several methods have been worked out for this purpose.

When starch or cellulose are heated in the presence of acids and water they are more or less rapidly broken down into dextrins and finally into dextrose. Some attempts have been made upon a commercial scale to hydrolyze the cellulose of sawdust and wood to dextrose, utilizing the sugar formed for yeast fermentation. The commercial manufacture of glucose from starch is based upon the acid hydrolysis.

A much more convenient method of preparing sugar from starch, preliminary to alcoholic fermentation, is by the use of malt. Malt is usually prepared from barley. This is steeped or soaked in warm water for twenty-four hours, drained and kept exposed to the air, thoroughly stirred and moist, until it germinates. Various mechanical devices are used for this purpose. In some cases large revolving drums containing the moist barley are used. In other cases large tanks with mechanical stirrers are employed, and in still others the malt is spread out on the floor and turned over with shovels by workmen. An examination of a barley grain, or the grain of any grass, will show that the young plant or germ lies on one side near the base of the starch or endosperm of the seed. The major portion of this germ is made up of a comparatively large shield-shaped organ called the scutellum. When the seed is soaked and started to germinate, certain cells of this scutellum form considerable quantities of the enzymes amylase or diastase. As the plant begins to grow this diastase diffuses out into the endosperm, transforming the starch into soluble sugars, which may be absorbed by the growing plantlet and used as food. The maltster has learned to recognize that stage in

the sprouting of the barley grain in which there has developed the maximum amount of diastase together with the minimum amount of transformation of starch. The sprouts are then broken off and the grain dried. This is termed the malt and is usually ground before use. The malt contains not only enough amylase or diastase to transform the starch present but much greater quantities as well. When ground malt is mixed with warm water the diastase goes into solution and rapidly transforms the starch present into the sugar maltose. If malt is added to any solution of boiled starch, there will be rapid transformation of practically all the starch present into maltose. For example, corn meal, rice meal, or boiled potatoes, when mixed with malt, soon dissolve and become sweet, due to the development of the malt sugar. Malt is the most common of the agencies employed for the saccharification of starch, preliminary to alcoholic fermentation, or when such transformation is required for other purposes.

In the commercial manufacture of alcohol for industrial purposes, cheaper methods for saccharifying starch have been discovered. Certain species of molds, particularly certain members of the genera *Aspergillus* and *Rhizopus*, are known to secrete large quantities of the enzyme amylase. These molds may be grown in the laboratory upon suitable sugar media, and the spores collected in large quantities. The material to be saccharified is usually boiled to soften it. Most frequently starch from rice, potatoes, or corn, is employed for this purpose. When sufficiently cooled, the spores of the *Aspergillus* or *Rhizopus* are introduced and mixed through thoroughly. Frequently air is bubbled through the mass to facilitate rapid growth of the mold mycelium. This soon penetrates to all portions of the mass and at once begins to excrete the enzyme amylase. Within a few hours or days, the whole mass of starch has been liquefied and transformed into sugar. Yeast can then be

added and the sugar fermented to alcohol and carbon dioxide.

Alcoholic Fermentation of Sugar Solutions.—The juices of many fruits contain sufficient quantities of sugar, usually dextrose, levulose, sometimes cane sugar, so that they furnish a natural medium for the growth of yeasts. In fact, yeasts in nature apparently grow for the most part upon the surfaces of bruised and diseased fruits. They are probably transported from one fruit to another by the agency of flies and various sucking insects. It is apparent, therefore, that when juice is pressed from fruits it usually contains a considerable inoculation of yeasts. The juices of fruits likewise usually contain considerable quantities of acids, particularly malic, citric, and tartaric. The hydrogen ion concentration in consequence is so high that most species of bacteria are inhibited from growth. This acidity is sometimes intensified by the wine manufacturer by the so-called process of sulphuring. In this process fumes of SO_2 are introduced, or compounds readily yielding SO_2 are added. The yeasts most commonly present on the surfaces of fruits, and which develop in their juices, belong to the group frequently termed *Saccharomyces ellipsoideus* because of the ellipsoid form characteristic of the cells.

Wines are prepared from the juice of the grape, *cider* from the juice of the apple, and *perry* from the juice of the pear. These may in turn be utilized for the manufacture of vinegar. It should be noted that alcoholic fermentation is essentially an anaërobic process. If any of these juices are left exposed to the air, the acetic bacteria will ordinarily begin to grow, transforming the product into vinegar. The juice of the century plant or agave in Mexico is fermented to produce *pulque,* and in certain tropical countries the juices of certain palms are fermented for the production of *palm wine.*

As previously noted, most of the common yeasts do not

possess the power to ferment lactose. A few species are known, however, which do have the power of developing lactase, therefore they are capable of fermenting milk. Alcoholic milk beverages have been prepared in many countries. The alcoholic fermentation in this case is usually accompanied by the formation of lactic acid as well, the product being a type of sour milk with a low percentage of alcohol.

Beer and *ale* are prepared by soaking malt in water to allow the action of the diastase upon the starch, transforming it into the sugar maltose. Certain proteolytic enzymes at the same time dissolve portions of the protein content of the malt. The solution is termed *beerwort*. To this, both in order to control undesirable fermentation and to impart a desirable flavor, an extract of hops is added. This hopped wort is then placed in large fermenting vats and yeast added, usually in pure culture. Fermentation proceeds until the desired concentration of alcohol has been secured. When fermentation is complete, the beer is clarified and put in bottles or kegs for distribution. The character of the product depends in large measure upon the kind of yeast used, particularly upon the use of bottom or top yeast.

Preparation of Distilled Liquors.—By a process of distillation the percentage of alcohol present in the final product may be greatly increased. *Rum* is prepared by fermenting molasses, the resulting alcoholic solution being distilled and flavored. *Brandy* is prepared by the preparation of wine or cider. *Whisky* is prepared by the inoculation of a mash made of malt and either ground grains or potatoes, which is then inoculated with yeasts. When the alcohol content has reached a sufficient degree of concentration and the fermentation is complete, the alcohol is distilled over and the product aged in suitable containers.

Manufacture of Commercial Alcohol.—The primary requisite for the manufacture of alcohol on a commercial

scale, is a cheap source of carbohydrate. Attempts have been made to hydrolyze the cellulose of wood and other materials, and this is being carried on on a commercial scale in some of the southern states. Usually, however, potatoes or cornstarch constitute the cheapest available carbohydrate. This is hydrolyzed either by the use of malt or of certain of the molds already described. It is then inoculated with yeast and when fermentation is complete, the alcohol distilled over.

Alcoholic Fermentation in Bread Making.—Many different leavening agents have been utilized in the preparation of bread. When wheat flour and water are mixed in proper proportions, the gluten of the flour forms a sticky or pasty mass, taking up most of the water. This is made porous by the introduction of minute gas bubbles, usually of carbon dioxide. This gas is, generally, on a commercial scale, generated by the growth of yeasts. The yeast used in bread making is of the same general type as that employed in the manufacture of beer. It will be recalled that yeast does not have the power to produce diastase, therefore fermentation induced by the yeast cannot take place until sugar is supplied. Flour contains small quantities of diastase and when mixed with water there is sufficient formation of sugar to allow of some growth of yeast. In commercial bread manufacturing, however, there is frequently added sugar, either maltose, dextrose, or sucrose, to increase the rapidity of the yeast development. Frequently also various chemicals are added to act as yeast stimulants. Certain salts of ammonia and of phosphoric acid increase materially the rapidity of yeast growth, and the production of alcohol and carbon dioxide. When, as the result of the growth of the yeast and proper kneading and mixing, the gas bubbles of carbon dioxide have been sufficiently distributed throughout the dough, it may be molded into form and baked. This process drives off most of the alcohol, coagulates the protein

or gluten, and increases at the same time the size of the gas bubbles.

In some cases bacteria have been substituted for yeast. Certain organisms belonging to the genus *Bacterium* are known to be able to split starch with the formation of carbon dioxide and hydrogen. "Salt-rising bread" is made by utilizing microörganisms of this type.

In order to prevent rapid deterioration of the bread after baking it is necessary that a certain amount of acid be present in the dough. This is usually formed as a result of the growth of certain lactic acid bacteria. Occasionally the acid is added by bakers as such. Proper observance of the acidity improves the texture of the loaf and renders impossible the development of ropy or stringy bread.

Alcohol Production in Other Foods.—More or less alcoholic fermentation may be incident to manufacture by fermentation of certain articles of food for man and domestic animals. During the process of fermentation of sauerkraut and in the preparation of ensilage more or less alcohol may be formed. Usually, however, the lactic bacteria outgrow the yeast, and alcoholic fermentation is secondary.

The Commercial Preparation of Yeast.—Originally it was customary for the housewife or the baker to carry along his own culture of yeast. Such a culture was ordinarily prepared by inoculating a mixture of potato water, sugar, hops, salt, and sometimes other ingredients, with a little of a preceding batch of the material. The culture consisted of a mixture of alcohol-forming yeasts and lactic acid bacteria. Such a home-made culture is, in the United States, usually termed a *starter*. In Great Britain it is usually called a *barm*.

In the commercial manufacture of yeast, it is necessary to grow large quantities of the yeast plants, free them from the material in which they have been growing, and press them into cakes. This is usually accomplished by seeding a

sugar solution with a suitable type of yeast and small quantities of lactic acid bacteria. Usually air is bubbled through the fermenting mixture. The yeasts multiply rapidly. They are removed from the liquid in which they are growing, either by sedimentation or by centrifugation in a machine somewhat resembling a milk separator. The yeast cells are then washed, again separated and pressed into cakes. In the so-called compressed yeast a comparatively small amount of starch will be present. In the dried yeast cakes the yeast is mixed with corn meal, or similar absorbent material, pressed into cakes and dried.

Development of Fusel Oils in Alcoholic Fermentation.—Important by-products in alcoholic fermentation are the fusel oils, consisting primarily of isoamyl alcohol. Formerly it was supposed that these were derived from the sugar, but more recent study has shown these to be derived from the amino acids developed from the digestion of proteins. Isoamyl alcohol is developed from isoleucine.

CHAPTER XVI

LACTIC ACID FERMENTATION—THE GENERA LACTOBACILLUS, STREPTOCOCCUS AND BACTERIUM

Lactic acid is produced in nature commonly as the result of the action of various microörganisms upon carbohydrates; it is also found in small quantities in tissues of the body as a result of muscular activity. Lactic acid fermentation is the most common change observed in milk, and fermentation of this type is most important in the development of the acid so necessary in the preservation of certain foodstuffs such as silage, sauerkraut, pickles and other fermented foods.

The exact chemistry of the formation of lactic acid has not been adequately explained. It is generally assumed to be due to the activity of an intracellular enzyme, the so-called lactacidase. The transformation does not require the presence of free oxygen, that is, it is essentially anaërobic. The change is one brought about by the organism as a means of securing energy. It is quite possible that the chemical transformation of sugar into lactic acid occurs in several steps. Disregarding the intermediate products, however, the reaction may be written:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} = 2\underset{\text{Lactic acid}}{CH_3 \cdot CHOH \cdot COOH}$$

It will be noted that the lactic acid molecule contains one asymmetric carbon atom. There are, therefore, two lactic acid forms, a levo acid and a dextro acid, the former turning the plane of polarized light to the left, the latter to the right. The kind of lactic acid developed in a particular

fermentative process apparently depends very largely upon the kind of organisms bringing about the change, the carbohydrate fermented and the temperature. Very frequently the acid formed is of the inactive type, that is, it consists of an equal mixture of the two acids, levo and dextro.

Lactic acid is the principal fermentative product of many kinds of bacteria belonging to the genera *Streptococcus* and *Lactobacillus*. Bacteria belonging to other groups, however, may sometimes produce small amounts of lactic acid associated with larger amounts of succinic, acetic, butyric or propionic acids.

Organisms Producing Lactic Acid

The organisms producing lactic acid primarily belong for the most part to the genera *Streptococcus* and *Lactobacillus*. Organisms producing smaller amounts of lactic acid and larger amounts of volatile acids, proteolytic ferments, etc., in general belong to the genera *Bacterium* and *Staphylococcus*.

The Genus Streptococcus.—There is much discussion in the literature relating to the souring of milk as to whether lactic acid production in milk is usually due to the presence of streptococci or of short rods occurring in chains. This has given rise to considerable confusion in names. It seems, however, that the organisms frequently alluded to in literature as *Bacillus lactis acidi* or *Bacterium lactis acidi* are for the most part, at least, streptococci and may be included under the species name, *Streptococcus lacticus*.

Another source of confusion in discussions of the streptococci as lactic acid producers has been the fact that the first species of *Streptococcus* studied, *S. pyogenes*, as well as many other species since described, are found associated with disease in man and animals. This led at one time to recommendation for the condemnation of milk which was found upon microscopic examination to show chains of cocci. It seems to

be well established that at least one, perhaps several, species of nonpathogenic streptococci are the most common organisms instrumental in bringing about lactic acid fermentation of milk.

Streptococcus lacticus has spherical cells sometimes slightly elongated, occurring in chains of greater or less length. It is Gram-positive, does not produce spores and is nonmotile. Apparently there are some strains, perhaps distinct varieties, capable of producing capsules. Certain of these capsulated types are responsible occasionally for the development of ropiness in milk, and for the sliminess experienced in certain cultures or starters. *Streptococcus lacticus* grows well, but never luxuriantly, on many of the laboratory media. Its growth is greatly increased in amount by the presence of suitable sugars which it may ferment. Media prepared from whey are frequently found to be useful. Lactose agar plates poured from souring milk will show the organisms developing as small pin-point colonies, frequently lying somewhat below the surface of the medium. If litmus or some other suitable indicator is present, the medium surrounding the colony will be noted to have become intensely acid. Upon agar slants, the colonies are usually more or less separated or discrete, at first scarcely visible, and at last dewdroplike in appearance. In ordinary broth the organism does not grow well unless sugar is present. Usually the medium clears rapidly by sedimentation. Gelatin is not liquefied.

The common sugars, dextrose, sucrose and lactose, are all fermented with the production of lactic acid but never with the formation of gas. There is some confusion in the literature as to the type of acid produced, but pure cultures in certain sugars, at least, frequently produce the dextro type only. When grown in milk sufficient acid is usually formed from the lactose (.5 to 1.25 per cent) so that coagulation or development of an acid curd occurs. The curd so formed is

usually smooth, free from gas bubbles, has a pleasant acid flavor and with little or no tendency to shrink and expel the whey. Its optimum growth temperature, apparently, is about blood heat, but it grows well (particularly in milk) at room temperatures and slowly at the temperature of the ice chest. Apparently there are strains of this organism which can resist the ordinary temperatures used in pasteurization. This is evidenced by the fact that milk which has been pasteurized in commercial plants usually retains sufficient numbers of these organisms in the living condition so that it sours.

The Genus Lactobacillus.—The bacteria of this genus are all Gram-positive, nonspore-producing rods developing commonly in milk and various fermented foodstuffs. A few species have been reported from the alimentary tract of man and animals. The group is sometimes known as the group of *high acid bacteria* because certain of the species will grow in a medium having a higher hydrogen ion concentration than will permit the growth of most bacteria, even of the *Streptococci.* Some of the species are relatively

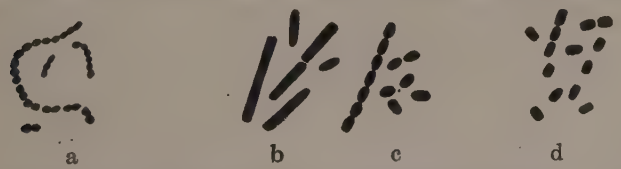

FIG. 50.—LACTIC ACID BACTERIA. A. *Streptococcus lacticus.* B. *Lactobacillus bulgaricus.* C. *Lactobacillus lactus acidi.* D. *Bacterium aërogenes.*

short rods. Most of them, however, are decidedly elongated, sometimes almost filamentous. The shortest rod forms apparently intergrade with the *Streptococci;* it is accordingly sometimes difficult to differentiate between *Streptococcus lacticus* and the *Lactobacillus lactis acidi.*

One of the best known species belonging to this group is the *Lactobacillus bulgaricus.* The organism was first

described by Metschnikoff who, when traveling through southern Russia and the Balkan countries, was much impressed by the large quantities of fermented milk and sour milk beverages used by the peasant classes as food. He also reached the conclusion that the peasants of this region were particularly long lived. He attributed this longevity to the large use of soured milk in the diet. He made a study of the favorite milk beverages and succeeded in isolating the organism chiefly responsible for the lactic acid formation. Inasmuch as it was isolated from the Bulgarian soured milk, it was given the specific name *bulgaricus*. The organism is a relatively large rod, sometimes filamentous. The cells are usually .5 to 1μ in diameter, and 2 to 3μ in length. In old cultures, however, some of the rods may be many microns in length. While the organism is definitely Gram-positive in young cultures, smears made from old cultures may show a mixture of Gram-positive and Gram-negative rods. Single cells may sometimes show a portion retaining the Gram stain and another portion decolorized and taking the contrast stain. This organism does not grow very readily upon the ordinary culture media, although its development in milk at the right temperature is abundant. The colonies produced in agar resemble a tiny mass of wool. The thread-shaped rods will be seen to penetrate the medium in all directions. A stab culture in whey agar or lactose agar gives a fir-tree appearance. *Lactobacillus bulgaricus* grows best at temperatures above blood heat, the optimum being 42° to 45°. Growth is slow at room temperatures. The total amount of acid formed is usually somewhat greater than produced by the *Streptococcus lacticus*, sometimes as much as four per cent being observed. Inasmuch as growth evidently continues in these relatively strong solutions of acid, the organism is termed *acid tolerant* or *acidophilous*. The curd produced in milk is usually somewhat slimy, smooth, and does not tend to contract and expel the whey.

The organism grows best under anaërobic conditions, better, therefore, below the surface of the medium than at the top.

Lactobacillus caucasicus and certain related species have been isolated from cheese and are probably important in the ripening process. Still other forms have been isolated from fermenting silage, sauerkraut, and other fermented foods. Many of them will grow at moderate temperatures or room temperatures and a few of them apparently possess the power of producing gas, particularly carbon dioxide.

Bacteria belonging to the genera *Bacterium,* and *Staphylococcus* are also capable of producing some acid in milk. Members of the former genus are important in development of lactic acid in certain foodstuffs.

The Genus Bacterium.—The organisms belonging to this group are all Gram-negative nonspore-forming rods, which develop readily upon the ordinary culture media, and are usually either aërobic or facultative. The most important forms are *Bacterium aërogenes* from milk and several species associated with the fermentation of sauerkraut and of silage. The organisms of this group, when present in milk, are usually termed the abnormal and undesirable lactic acid bacteria. Some of them, however, may be desirable in the fermentation of other food products. Most of the bacteria belonging to this group, particularly those of importance in milk and the dairy industry, have the power of producing gas from sugars. They develop also certain of the volatile acids in addition to the formation of lactic acid.

Bacterium aërogenes grows well on ordinary culture media. The colonies on agar or gelatin are much larger and more inclined to be slimy than those of the organisms belonging to the genera *Streptococcus* or *Lactobacillus.* For the most part they grow best at blood heat. Most of them also develop well at lower temperatures. When suitable carbohydrates which they can ferment are present, the organisms will develop anaërobically. In the absence of

such sugars they are aërobic. Dextrose, sucrose, maltose, and lactose are all fermented with the development of acid and gas. A total of one to one and one-quarter per cent of acid may develop in milk, but as mentioned above, it is only in part lactic acid, acetic and other volatile acids being present. Usually the lactic acid formed is levorotatory. The gas developed is a mixture of hydrogen and carbon dioxide. When grown in milk the curd produced is usually torn by gas bubbles and shrinks, expelling a considerable proportion of the whey. When present in milk in unusually large quantities, such organisms may interfere with the production of the best quality of cheese.

Technical Utilization of Lactic Acid Fermentation

Associative Action.—It has frequently been observed, particularly in the development of bacteria in milk, that mixed cultures are able to bring about changes which pure cultures of the constituent organisms could not produce. In some cases there is an increased production of acid. In other cases the type of the change in milk is altered materially. It will be noted below that several of these associative actions, particularly those among the desirable bacteria, bring about changes of considerable economic importance. Inasmuch as bacteria rarely occur in pure culture in nature, the spontaneous changes brought about in milk and the various fermented foods must, therefore, in general be the result of associative action.

Lactic Acid Bacteria in Milk

Milk when freshly drawn, practically always contains some lactic acid bacteria, usually streptococci and lactobacilli. These may have come in part from the udder, from the coat of the cow and very largely, usually, from the milk utensils. When the milk is allowed to stand, particularly in

a warm place, they multiply very rapidly and usually overgrow other kinds of bacteria which may be present. Sugar is rapidly transformed into lactic acid. The total number of the lactic acid bacteria developing will usually run into the hundreds of millions per cubic centimeter. Eventually sufficient lactic acid develops to curdle the milk and effectually stops the growth of the bacteria. *Streptococcus lacticus* is usually inhibited first. Certain species of lactobacilli may go on growing, producing larger quantities of lactic acid, after the inhibition of the streptococci.

When members of the genus *Bacterium,* particularly *Bacterium aërogenes,* are present in sufficient quantities to modify the course of fermentation, the curd developed, as noted above, is usually broken and tends to shrink.

Soured Milk Beverages.—An artificial buttermilk (frequently sold at soda fountains) is prepared by inoculating milk with a previously soured sample of milk or with a pure culture of lactic acid bacteria. In most cases the resultant souring is produced by an organism of the *Streptococcus lacticus* type. When the milk has curdled it is beaten or churned, so that the casein becomes finely divided. In some respects it is preferable to the true buttermilk as a beverage because of the greater uniformity in flavor and texture secured.

Milk sherbets (as lacto) are frequently prepared from milk soured in this manner, flavored with fruit juices, and frozen.

In some cases, the organisms used for souring the milk belong to the genus *Lactobacillus,* usually *Lactobacillus bulgaricus.* As noted above, milk soured by this organism is somewhat more slimy and has a smoother curd than milk soured with *Streptococcus lacticus* or related organisms. The vogue of the so-called Bulgarian soured milk owes its origin to the work of Metschnikoff. This observer came to the conclusion that the consumption of soured milk bev-

erages of this type is decidedly conducive to longevity because it induced the development of a lactic acid flora in the large intestine, and there was, in consequence, little or no development and consequent absorption of poisonous putrefactive substances from the colon. Acting upon this suggestion pharmaceutical houses have prepared tablets consisting usually of mixtures of milk sugar and dried cultures of *Lactobacillus bulgaricus*. More recent work seems to indicate that while soured milk of this type is an excellent food, nevertheless, the unusual health-promoting qualities ascribed to it by Metschnikoff have scarcely been confirmed by the facts as they have developed. Bulgarian soured milk undoubtedly has a place in the dietary of invalids and convalescents.

Soured milk beverages have been used apparently since ancient times practically all around the shore of the Mediterranean both in Africa and Europe, about the Black Sea and the Balkan countries, in Greece, in southern Russia, the Caucasus and parts of India. The methods of manufacture differ materially with the locality. In some cases the milk is subjected to a preliminary heating and is then inoculated by introducing a small amount of a previously fermented batch of milk. It is then kept in a comparatively warm place. This encourages the growth of bacteria belonging to the group of lactobacilli, particularly *Lactobacillus bulgaricus*. A soured milk of good flavor and comparatively high acid content is thereby secured.

In certain districts dried kernels, termed kefir grains, are introduced into the milk which is to be fermented. When examined microscopically these kefir grains are found to be made up of gelatinous masses of bacteria with yeast cells embedded throughout. When introduced into milk a combined lactic acid and alcoholic fermentation occurs. The resultant beverage is somewhat acid and effervescent, containing a low percentage of alcohol. It should be observed

that the yeasts present are those capable of producing the enzyme lactase, therefore, of fermenting lactose.

Sour Curd Cheese.—When milk that has been soured is heated, the curd contracts, the whey is expelled and may readily be drained away. The curd may then be worked or molded, or mixed with butter or various flavoring materials, and converted into a sour milk cheese usually termed Dutch cheese or cottage cheese. This type of cheese is not usually subjected to a ripening process.

The Use of Starter.—While cream may be churned into butter without any preliminary fermentation and while butter from this sweet cream is preferred in certain localities and for certain purposes, nevertheless, most of the butter sold in the United States is churned from cream which has been allowed to ripen, that is, it has been allowed to undergo a process of acid fermentation. The flavor and aroma of the butter will depend very largely upon the type of organisms which have been growing in the cream. The butter fat absorbs considerable amounts of dissolved substances present in the fermenting cream, it is, therefore, highly desirable that these should be of the proper character. If batches of cream are allowed to ripen spontaneously without the addition of any starter, there is apt to be a marked lack of uniformity in the product. A much better and more uniform result is secured by inoculating the cream which is to be ripened with a considerable amount of desirable lactic acid bacteria. Such organisms are sold under the name of commercial starters. It is generally assumed that they are pure cultures, or practically pure cultures, of *Streptococcus lacticus* or some very closely related organism. This is introduced into pasteurized milk and allowed to grow until the milk has reached a satisfactory state of acidity. With this a larger bulk of milk is inoculated and finally the whole mass used as a starter for the cream which is to be churned. A heavy inoculation with

the desirable microörganisms and their growth products usually overwhelms undesirable types of bacteria and leads to the development of a satisfactory product.

Recent studies have made it evident that the changes brought about in a satisfactory starter are not the results simply of the growth of *Streptococcus lacticus*. Another organism closely related to it must ordinarily grow with it. Cream which has been ripened by means of a pure culture of the ordinary *Streptococcus lacticus* does not produce butter with as satisfactory a flavor and aroma as that produced by cream which has been inoculated with a mixture of these organisms. The ripening produced by a starter is, therefore, an excellent example of associative action. The true *Streptococcues lacticus* apparently produces very little change in milk or in cream other than the development of the pure lactic acid from lactose. The associative organism, also a coccus (either *Streptococcus citrovorus* or *Streptococcus paracitrovorus*) can by its own growth bring about very little change in milk. When grown in the presence of *Streptococcus lacticus*, however, the activity of this organism is greatly stimulated and small amounts of volatile acids are developed. It is to the absorption of these and perhaps of other growth products that the butter owes its characteristic aroma and flavor.

Effects of Pasteurization upon the Souring of Milk

Some of the earlier opponents of pasteurized milk urged that the process of pasteurization, if properly performed in a manner to eliminate all disease-producing bacteria in the milk, would likewise kill all of the lactic acid bacteria, and that the only organisms remaining alive would be spore-producing bacilli and clostridia. These would then begin to grow, after the milk had cooled, and bring about putrefactive changes. However, the work of Ayers and his associates in the Dairy Division of the Bureau of Animal

Industry seemed to indicate that commercially pasteurized milk practically always contains resistant strains of *Streptococcus lacticus* which will bring about normal souring, and that putrefactive changes in pasteurized milk are little if any more common than in milk which has not been pasteurized.

Secondary Changes in Soured Milk.—When soured milk is allowed to stand, lactic acid formation eventually ceases and very little change occurs in the milk for a considerable period of time unless air is allowed access to it. This has been taken advantage of in certain districts in northern Europe to preserve meat for a time in soured milk.

In certain of the Scandinavian countries, particularly in Norway, milk is kept from the winter months for use in the home during the summer months while the cattle are away in the mountain pastures. The milk is first heated and then stored in casks after inoculation with a suitable starter. The organisms present are lactic acid bacteria and a species capable of producing a considerable degree of viscosity. This ropy milk will keep for a considerable period of time if air is excluded.

When air gains access to soured milk, certain aërobic forms, particularly certain molds such as *Oïdium lactis*, begin to grow upon the surface. These organisms rapidly oxidize the lactic acid to carbon dioxide and water, thus decreasing the acidity of the solution. At the same time certain of the molds produce proteolytic ferments which bring about partial or complete digestion of the casein. When the acidity of the solution has been sufficiently decreased, certain of the aërobic and anaërobic spore-producing bacteria find conditions favorable for development and bring about proteolytic or even putrefactive changes.

Lactic Acid in Cheese Manufacture.—Rennet curd cheeses are in part ripened through the activity of lactic

acid bacteria. Usually rennet acts rather more rapidly and more satisfactorily upon milk in which a slight amount of lactic acid has developed. Bacteria of the group *Streptococcus lacticus* and particularly *Lactobacillus caucasicus* and related species rapidly transform any residual lactose in cheese into lactic acid. The other changes incident to cheese ripening will be discussed in another connection.

Lactic Acid in Food Preservation

Ensilage.—Ensilage or silage is prepared by chopping or shredding various green foods and packing them compactly into an air-tight structure termed a silo. In general the food plants most used for a silo are those which contain considerable quantities of sugar when green and of starch when mature; such, for example, are corn and sorghum. Other fodder plants may be ensiled but unless the content of sugar or starch is sufficiently high, abnormal or putrefactive changes may occur. This may be obviated by mixing with corn or with sorghum. Such, for example, would be silage made from mixtures of Indian corn and soy beans or cow peas.

The most essential change occurring in the ensilage is the transformation of a part of the carbohydrates present into lactic acid. Carbon dioxide, acetic acid, traces of butyric and other acids and sometimes alcohol may be formed. The lactic acid accumulates in quantities sufficient to prevent the development of putrefactive or other undesirable microörganisms, providing conditions are satisfactory.

For proper fermentation in the preparation of silage it is necessary to have, first, material properly prepared by shredding; second, the ensilage packed tightly into an air-tight container or silo so as to leave as little air space as is practicable; third, the presence of an optimum amount of moisture, and fourth, the presence of suitable bacteria.

Most of the difficulties in the preparation of satisfactory ensilage are due to defects of one of the first three factors. The suitable lactic acid bacteria are usually present. However, certain investigators have found increased uniformity of results when the silage is inoculated with suitable mixtures of lactic acid bacteria at the time of preparation.

Usually the materials placed in the silo are green, that is, they contain many living cells. Among the first changes which occur, therefore, in the silo are those due to the activity of these living cells and of the enzymes present in them. Certain of the earlier investigators of silage concluded that these changes were by far the most important. More recent investigations, however, have shown that the bacteria are responsible for most of the fermentative changes. Yeasts, which are usually present, may bring about some alcoholic fermentation. The lactic acid bacteria, however, usually overgrow and outgrow the other organisms present and produce sufficient lactic acid to inhibit their development completely. Eventually the growth of the lactic acid bacteria is likewise stopped, and the food material or silage will then usually keep almost indefinitely, providing air does not gain access.

A considerable rise in temperature may take place in the silo during the process of fermentation. If this increase is excessive it usually indicates that there is not sufficient moisture present and the material has not been tightly enough packed. The lactic acid fermentation does not give rise to quantities of heat comparable with the amount developed during oxidative changes occurring due to the growth of molds and bacteria in the presence of atmospheric oxygen. The surface layers of silage usually become quite warm, for air can penetrate to the depth of several feet. These, therefore, can be preserved only by covering the surface carefully with something which will exclude the air.

If air gains access to the silage, molds will soon develop and oxidize the lactic acid to carbon dioxide and water, and putrefactive changes may then be brought about. Moldy silage, of course, has lost much of its feeding value and is dangerous particularly for horses.

The organisms responsible for bringing about the fermentation are members of the genera *Bacterium* and *Lactobacillus* for the most part. Organisms closely resembling if not identical with *Lactobacillus bulgaricus* and *Bacterium aërogenes* are usually abundant. The characteristic odor of good silage is due to the presence of certain volatile acids and to the formation of esters with the traces of alcohol present.

Carbon dioxide may be produced in considerable amounts during the fermentation of the silage. Care should be used, therefore, in entering a silo which is partly filled with silage. It may be recalled that carbon dioxide is heavier than air and that there are numerous cases of asphyxiation on record where care has not been used to see that the silo is free from this gas in injurious quantities. The danger would be particularly great in a silo where the process of filling has been interrupted and men enter the silo a few days later to tramp down new silage as it enters.

The fact that the fermentation is essentially anaërobic and that there are organisms present, such as members of the genus *Bacterium,* which produce not only carbon dioxide but hydrogen as well, leads to strong reducing action. Considerable amounts of sugar are transformed into the polyatomic alcohols. Fructose especially is converted into mannitol ($C_6H_{14}O_6$).

Sauerkraut.—Sauerkraut is prepared by shredding or slicing cabbage, mixing with salt and pressing firmly into casks or vats. The sugary juices of the cabbage leaves together with the salt soon form a liquid which covers the mass. Lactic acid fermentation, brought about largely by

microörganisms belonging to the genus *Bacterium,* perhaps secondarily by organisms of the genus *Lactobacillus,* ensues. Gas is given off in considerable quantities. Among the gases developed are considerable quantities of those containing sulphur, particularly hydrogen sulphide. It may be noted that cabbage contains appreciable amounts of glucosides containing sulphur. These are largely broken down during the process of fermentation. Finally the lactic acid content of the brine rises to a point which will inhibit the growth of all microörganisms which are not aërobic. Sauerkraut thus prepared will keep for a considerable length of time if not exposed to atmospheric oxygen. When so exposed, however, it rapidly molds, the lactic acid is oxidized and decay occurs.

Other Fermented Foods.—Sweet corn, beets, and some other foods are occasionally preserved in a manner similar to saucrkraut. When mixed with a suitable amount of salt and pressed firmly into jars or barrels, lactic acid fermentation will occur and effectually preserve the food material as long as it is not in contact with the air.

Lactic Acid in Yeast Manufacture.—A medium somewhat acid is usually required in the production of commercial yeast in order to prevent the development of undesirable microörganisms. Certain bacteria, members of the genus *Lactobacillus,* are usually introduced into the vats preliminary to the growth of yeasts in the commercial manufacture of compressed yeast or dried yeast cakes.

Lactic Acid in Bread Making.—The bacteria present in yeast cakes and in flour usually bring about the development of some lactic acid during the leavening process in the bread. The texture of the loaf and its flavor are in part dependent upon the development of the lactic acid. Furthermore, the keeping quality of the bread is directly related to it. If sufficient lactic acid does not form during the leavening process the bread is very apt to become ropy

when kept in a warm place after baking. Commercial bakers not infrequently add lactic acid to the dough to insure its presence in sufficient quantities.

So-called "salt-rising" and certain types of "sour dough" bread are leavened by the action of organisms belonging to the genus *Bacterium*. They produce small quantities of lactic acid and considerable quantities of gas, particularly carbon dioxide and hydrogen. They thus replace yeast in forming the gas bubbles essential to the leavening of the loaf.

CHAPTER XVII

ACETIC, BUTYRIC, OXALIC AND CITRIC FERMENTATION—THE GENERA ACETOBACTER, BACTERIUM, CLOSTRIDIUM, ASPERGILLUS AND CITROMYCES.

Many different acids have been described as produced by microörganisms. Among the most important in this list are formic, acetic, propionic, butyric, valerianic, succinic, lactic, oxalic, and citric. Of these, lactic acid has already been discussed. Acetic, butyric, oxalic and citric acids are of considerable economic importance.

Acetic Fermentation

Many microörganisms are known which are capable of producing acetic acid. Comparatively few of these, however, are of economic significance. Acetic acid production is most important in the manufacture of vinegar for use as a condiment, although large quantities are produced by bacterial fermentation for the manufacture of ingredients (particularly amyl acetate) used in making certain explosives. Small quantities of acetic acid are also produced in many fermented foods such as sauerkraut, ensilage, and fermented pickles.

Types of Acetic Acid Fermentation.—Bacteria producing acetic acid may be divided into two groups; those which bring about the change under aërobic conditions, oxidizing alcohol to acetic acid; and those which bring about fermentation of sugars under anaërobic conditions, acetic acid being one of several of the products.

Alcoholic solutions, such as wine, beer or cider, when allowed to stand open to the air soon become covered with a growth popularly termed *mother of vinegar*. This is com-

posed largely of microörganisms which rapidly oxidize the alcohol to acetic acid. The enzyme produced by the organisms capable of bringing about this change has not been isolated, but it probably may be regarded as an intracellular enzyme belonging to the general group of the oxidases. The reaction may be indicated as follows:

$$C_2H_5OH + O_2 = CH_3COOH + H_2O$$

The bacteria which bring about the change under anaërobic conditions usually produce considerable quantities of butyric, lactic, succinic or other acids simultaneously with the formation of acetic acid. The changes are relatively complex and the process of the fermentation is not well understood; it cannot, therefore, readily be represented by a simple chemical equation.

Groups of Acetic Bacteria.—As previously noted, the bacteria producing acetic fermentation are either aërobic or facultative and anaërobic. Of the former group the organism *Acetobacter aceti* may be taken as a type. Of the latter group, organisms belonging to the genera *Bacterium* and *Clostridium* are worthy of mention.

The Genus Acetobacter.—To this genus of bacteria belong most of the forms capable of oxidizing ethyl alcohol to acetic acid under aërobic conditions. Many species of *Acetobacter* have been described but all are apparently closely related. They differ from each other slightly in morphology, in their ability to produce large or small amounts of acid and to attack various sugars. The "mother of vinegar" which forms on the surface of wine, cider or other alcoholic solutions when allowed to stand, consists for the most part of a tangled mass of bacteria belonging to this genus together with yeast cells instrumental in bringing about the preliminary alcoholic fermentation. All of the bacteria belonging to this genus are rod-shaped, usually relatively long and occur in chains. They are usually non-

motile and do not produce spores. For the most part, they grow readily upon suitable culture media and are obligate aërobes. Some produce such small quantities of acid that they are of no economic significance. Others produce the acid rapidly and in large quantities. Different species have been described from wines, from cider, from beer and from the so-called quick process of vinegar manufacture. The cells for the most part produce considerable amounts of slime and tend to form films on the surface of the medium. Involution forms, that is, cells showing unusual shapes such

FIG. 51.—ACETOBACTER. Normal rods and involution forms.

as clubs, branched forms, spheres, etc., are very common, particularly in the older films.

Acetobacter aceti.—This organism may usually be differentiated from related acetic bacteria by the fact that it produces a surface pellicle upon the alcoholic solution which can be easily broken up; that is, the development of slime is not so evident as in some other species. The slime from this organism is not stained by the use of a solution of iodine or of potassium iodide. The organism has usually been isolated from the surface of souring beer.

Another organism which has been described from beer vinegar is the *Acetobacter pasteurianum*. Some authors have also described it as one of the important agencies in the production of wine and cider vinegar. It develops as a membrane on the surface of the liquid, at first appearing

moist, later dry, and finally wrinkled, while the medium below the surface remains quite clear. The bacteria occur in chains, although greatly elongated filaments may be observed. This organism produces a much larger amount of slime than the *Acetobacter aceti,* and the slime turns blue when potassium iodide or iodine is added. The involution forms are particularly abundant. Upon beer-wort and beer-wort gelatin the organism grows readily. It has a high power of oxidizing alcohols to corresponding acids, ethyl alcohol being rapidly transformed to acetic acid, propyl alcohol to propionic acid, and dextrose to gluconic acid

$$(CH_2OH(CHOH)_4COOH).$$

Acetobacter orleanense is regarded as one of the most common causes of acetic fermentation in fruit juices such as the wines and ciders. It differs only in minor morphological and physiological characters from species of the *Acetobacter* previously described. It can produce acid from many sugars but best where it can oxidize alcohol to acetic acid. It may be noted that this organism, as well as other species of *Acetobacter* may continue the action beyond the point of complete oxidation to acetic acid, and may also, when the alcohol supply has been exhausted, oxidize the acetic acid to carbon dioxide and water. This makes it necessary to pasteurize or to seal vinegar from the air when it has been prepared, otherwise the same organisms which produced it will destroy it or cause it to "lose its strength."

The *Acetobacter schützenbachii* may be taken as a representative of the species which have been described in the so-called quick process of vinegar manufacture. While it grows readily upon ordinary culture media, it does not produce a heavy film upon the surface of liquids. It is, therefore, not an organism which would ordinarily bring about the transformation of cider or wine into vinegar by the slow process requiring film formation. The bacterial

cells are usually oval, sometimes elongated or pickle-shaped. Usually they occur in pairs though sometimes they are united into chains of different lengths. This organism develops rapidly upon the shavings used in the quick vinegar process and is very active in the oxidation of alcohol to acetic acid. As much as 11.5 per cent of acetic acid has been produced in alcoholic solutions by organisms of this type.

Vinegar Manufacture.—Vinegar may be defined as a solution of acetic acid (usually from 4 to 8 per cent, preferably about 5 to 6 per cent) developed by the fermentation of alcoholic solutions of various types. The origin of vinegar is ordinarily indicated by such phrases as "cider vinegar," "wine vinegar," "malt vinegar," "honey vinegar,' etc. The manufacture of vinegar will be discussed under the headings of manufacture in the home and on the farm and commercial manufacture.

Home Manufacture of Vinegar.—The materials most commonly used for the home manufacture of vinegar are cider or other fruit juices, honey, and artificial sugar solutions. The first step in the process is always an alcoholic fermentation, the transformation of the sugar, at least in large part, into alcohol and carbon dioxide. This change may be initiated by the introduction of yeast, but with the fruit juices this usually is not necessary, the native wild yeasts present being sufficiently numerous and active. For the manufacture of a good quality of vinegar it is necessary to have first, a sufficient exposure of surface of the liquid to the air to allow the abundant development of the surface film of acetic bacteria; second, the rapid transformation of the alcohol into acetic acid due to the presence of desirable acetic bacteria; third, maintenance of the material at a suitable temperature, and finally, some method of stopping the process upon the formation of the maximum amount of acetic acid and before microörganisms have transformed

any considerable proportion of the acetic acid into carbon dioxide and water, thus weakening the vinegar. For this purpose casks or barrels may be partly filled with cider or wine (preferably not more than one-half to two-thirds full at the most), placed upon their sides and an opening left for ingress of air. This opening should be closed with a layer of cheesecloth or gauze to prevent insects from gaining access. The process may be considerably hastened by adding some good vinegar or some "mother of vinegar," or best of all a culture of the most suitable acetic bacteria.

Washings from honey extractors and improperly ripened honey may be manufactured into vinegar of very fair quality by the same process.

Many housewives in recent years have manufactured vinegar in small quantities in the home by making use of what have come to be known popularly as "vinegar bees." These consist of dried, brownish, gelatinous lumps, varying from the size of a pinhead to a bean. When examined microscopically they are found to consist of a gelatinous mass of bacteria with many yeasts. When dropped into a solution of sugar, the yeasts at first multiply rapidly and bring about alcoholic fermentation. The bacteria soon begin to develop, produce the characteristic acetic film, and transform the alcoholic solution into vinegar. Homemade vinegar of this type is often prepared by the use of fruit juices, syrups that have been used in preserving fruits, from molasses, or even from cane sugar or honey. Some samples of vinegar of fair quality are prepared in this way, though usually the vinegar is not as good as that secured by the fermentation of cider. In most instances the acid content is relatively low, rarely reaching 4 or 5 per cent. It should not be used, therefore, for preservation of food materials requiring a high percentage of acid.

Commercial Manufacture of Vinegar.—The best commercial vinegars are manufactured from cider and wine,

though vinegar of good quality may be secured from beer-wort or malt extract. Much vinegar, however, is manufactured by the alcoholic fermentation of molasses or cheap syrups. In some cases a mixture of acetic acid secured from the destructive distillation of wood diluted with water and colored with caramel is sold as vinegar. This is generally prohibited by law as fraudulent.

One of the chief aims in commercial manufacture of vinegar is the quickening of the process. One of the earlier methods, and one still practiced, is the so-called Orleans method, resembling in many respects the method of vinegar manufacture already described as suitable for the home and farm. A cask is partly filled with a mixture of wine and good vinegar. It is allowed to stand until a satisfactory scum or membrane has formed over the surface and until the entire contents have been converted into vinegar. A definite amount of the vinegar, usually a gallon or more, is withdrawn and an equal amount of wine added, care being taken not to break the membrane covering the surface of the liquid. After the process is well started a portion of the contents of the barrel may be withdrawn daily or at other suitable intervals and equal amounts of wine added. The method has certain disadvantages. The membrane is apt to be broken and portions will settle to the bottom where the organisms composing it cease growing and decompose, adding to sediment and producing undesirable flavors.

Pasteur and his collaborators developed a so-called vat method of vinegar manufacture in which a suitable mixture of wine and good vinegar was poured into a relatively shallow vat and a light wooden lattice floated upon the surface. The membrane, consisting of acetic bacteria, soon developed and remained supported by the wooden frame. As soon as the material had been converted into vinegar a portion was withdrawn and an equal amount of unfermented material added. This withdrawal can be made

daily and a constant supply of vinegar thus secured. The process has the advantage of providing abundant surface area with consequent more rapid fermentation and it likewise preserves the acidifying membrane intact.

It may be readily deduced that the larger the relative area of the surface of the liquid exposed to the air, and the more rapid and thorough the aëration, the more rapid, under given conditions, will be the transformation of alcohol into acetic acid. This has led to the manufacture and development of various devices for hastening the manufacture of vinegar by exposing the liquid in very thin layers to the action of organisms in the presence of air, layers much thinner than those present in the Pasteur vat method. The device probably most used at the present time is the so-called quick or German method of vinegar manufacture. The apparatus consists essentially of a tall cask or wooden cylinder filled with beech-wood shavings. Beech-wood is chosen in preference to other woods because it contributes but little to the flavor of water coming in contact with it. The shavings rest upon a false bottom bored full of holes. An automatic sprinkling device is installed above the top of the shavings. These shavings are first inoculated with suitable bacteria, usually organisms of the type of *Acetobacter schützenbachii*. This may be accomplished by sprinkling the beech-wood shavings with a good quality of vinegar previously manufactured by the process or pure cultures may be used. The alcoholic solution to be fermented is sprayed or sprinkled over the top of the shavings and trickles down over their surfaces to the bottom and finally into a suitable receptacle. The surface area of the shavings is relatively large. The liquid flows down over them in very thin layers. The microörganisms find favorable conditions for growth inasmuch as air is constantly passing in at the bottom, up through the shavings and out at the top. A constant circulation of air is easily main-

tained because some heat is generated during the process of fermentation, giving rise to convection currents. The bacteria soon cover the surfaces of the shavings with a continuous film. Conditions are optimum, therefore, for the rapid oxidation of alcohol to acetic acid. After the apparatus is in working order and the shavings thoroughly inoculated and the rate of flow properly adjusted, the alcoholic solution may run in at the top in a continuous stream and vinegar be drawn off at the bottom.

Butyric Fermentation

The development of butyric acid (C_3H_7COOH) in fermentation is usually regarded as an undesirable change, although in some operations such as retting of flax and hemp, it may be desirable and even essential.

Butyric acid is produced in several distinct ways in various fermentative processes. Certain anaërobic bacteria ferment sugars (and in some cases polysaccharides) with the formation of butyric acid and gas, usually carbon dioxide and hydrogen. If the intermediate steps are disregarded the reaction may be indicated as follows:

$$C_6H_{12}O_6 = C_3H_7COOH + 2CO_2 + 2H_2$$

Lactic acid or the lactates may also be fermented by these organisms. The reaction in this case may be represented as:

$$2CH_3 \cdot CHOH \cdot COOH = C_3H_7COOH + 2CO_2 + 2H_2$$

In some cases glycerin is fermented with the development of butyric acid. The acid may also originate as a result of the hydrolysis of the fat butyrin.

Organisms Producing Butyric Fermentation.—Most of the bacteria responsible for butyric fermentation of carbohydrates belong to the genus *Clostridium;* in fact, probably most of the species of this genus produce more or less

butyric acid. Among these are the organisms which produce tetanus or lockjaw, blackleg in cattle, gaseous gangrene and malignant edema. Certain of the bacteria most important in bringing about putrefaction, such as *Clostridium putrificum,* are likewise important. The organism most commonly associated with butyric fermentation is *Clostridium butyricum.* It is a rod-shaped, Gram-positive, motile rod, producing spores, the cell during sporulation becoming swollen or spindle-shaped. It can be grown in ordinary culture media under anaërobic conditions. In the

FIG. 52.—CRYSTALS OF CALCIUM OXALATE IN MEDIUM IN WHICH *Aspergillus* IS GROWING

presence of sugars, particularly of dextrose, abundant gas formation and development of butyric acid occurs. The process is soon halted, however, by the increasing acidity of the solution unless some means is taken to neutralize the developing acid by the presence of some base, such as calcium carbonate.

The *Clostridium pectinovorum* has been repeatedly isolated from hemp during the process of retting by the wet method. This organism has markedly developed the power to dissolve the pectin from the middle lamella joining the cells and bundles of bast fibers together in the stems of plants. Pure cultures have been used to some extent in facilitating the process.

Formation of Other Volatile Aci..—Certain bacteria are capable of producing formic, propionic and valerianic acids from carbohydrates. These are less frequently met with and are not as important, therefore, as those bringing about acetic and butyric fermentations.

Oxalic Fermentation

Certain species of molds are known which are capable of producing oxalic acid from sugars. The most important of these belong to the genera *Aspergillus* and *Penicillium*. The process is essentially aërobic. The presence of the oxalic acid is easily demonstrated by growing the organisms in a medium containing some soluble salts of calcium. The insoluble calcium oxalate is precipitated. The crystals of this salt usually may be readily observed in an agar plate culture of such a mold.

Citric Acid Fermentation

Certain species of molds belonging to the genera *Citromyces* and the closely related genus *Penicillium* have been found to possess the power of transforming sugar into citric acid. The acid is produced in sufficient quantities so that patents upon this method of preparation have been taken out by commercial firms in Europe. Most of the citric acid on the market, however, comes from native fruits such as the orange and lemon and not from citric fermentation.

CHAPTER XVIII

FERMENTATION OF POLYSACCHARIDES AND FATS

The Gums—Hemicelluloses and Celluloses

Characteristics.—The gums, hemicelluloses and celluloses are the most complex of the carbohydrates known to occur in nature. They all have much the same empirical formula, in most cases represented by $(C_6H_{10}O_5)_n$. They differ in their action toward various reagents. The *gums* will swell in water and are soluble in hot water. The *hemicelluloses* may be softened and even put into solution by hot water. *Celluloses*, however, are soluble only in strong sulphuric acid and in ammoniacal copper sulphate. All may be broken down by hydrolysis with hot acids through a series of intermediate compounds to simple sugars having the formula $C_6H_{12}O_6$, or in a few cases to pentoses, $(C_5H_{10}O_5)$.

Distribution.—The *gums* occur commonly in nature as products of plant growth. The agar-agar used in the bacteriological laboratory is a gum derived from various members of the group of red or purple seaweeds. The gum tragacanth and the gum arabic of commerce are examples of gums produced by higher plants. Gums may frequently be observed upon certain trees, such as the cherry. The *hemicelluloses* are relatively abundant in certain seeds. The date seed, for example, consists for the most part of compounds belonging to this group. The true *celluloses* make up most of the cell wall of higher plants.

Fermentation.—It is evident from even casual observation in nature that cellulose and plant bodies rapidly decay and disappear, particularly when incorporated into the soil.

This change is brought about by the activity of microörganisms of many types. Some species apparently work best under anaërobic conditions. Others are essentially aërobic.

Several species of bacteria belonging to the genus *Clostridium* have been described as capable of bringing about anaërobic decomposition of cellulose. It is a matter of common observation that when dead plant tissues decompose under anaërobic conditions, as in the mud at the bottom of a pond, carbon dioxide, methane (CH_4) and hydrogen gas are evolved. It is found that during this process of decomposition there are developed compounds having higher percentages of carbon until, under the right conditions, practically the entire organic remains consist of carbon and hydrocarbon. This is the kind of change occurring in the process of peat formation. The plant roots, mosses and remains of stems buried under water gradually decompose, but residues high in carbon are left. It is probable that coal has originated in the same manner. Probably a portion at least of the humus in the soil is developed by the same general process, although aërobic bacteria probably play a considerable part in its manufacture.

A distinct group of organisms, either anaërobic or facultative forms, are of importance in bringing about cellulose digestion in the alimentary tract of herbivorous animals. The horse and the cow, for example, eat large quantities of cellulose. A study of the digestion occurring in these animals reveals that a part, at least, of this cellulose is digested and utilized. Careful search, however, has failed to show that herbivorous animals in general are capable of producing any enzyme which will digest cellulose. It is quite probable that microörganisms present in the paunch of ruminants and in the large intestine of the horse are responsible for much, if not all, of this fermentation and digestion. Many efforts have been made to isolate the organisms responsible, and although some species capable of

digesting cellulose slowly have been isolated, apparently the most important types have not thus far been found.

Many species of bacteria have been isolated capable of decomposing cellulose under aërobic conditions. Agar plate cultures containing finely granulated precipitate of cellulose, constitute a suitable substrate for the growth of such organisms. The bacteria produce an enzyme, cytase, which dissolves the cellulose in the area immediately about the colony and this area then becomes clear. Certain species belonging to the genus *Actinomyces* are also believed to be of importance in the soil. They are very common upon decaying plant roots, and are abundant in and on the decomposing straw of manure piles. Certain molds, among them many species of *Penicillium*, are known which actively digest cellulose under aërobic conditions.

The products resulting from the action of microörganisms upon cellulose have not been adequately investigated. Whether or not the cellulose is actually broken down into sugar, for example, before it is utilized by the bacteria or transformed further, is not known. It seems fairly certain, however, that in some cases, at least, the intermediate products of the decomposition of the cellulose are utilized more or less directly and sugar is not formed at all.

Organisms capable of bringing about cellulose fermentation are of great importance in nature, particularly in the soil. By the changes they bring about they make available sources of energy to many other types of soil bacteria. Their functions will be noted later in connection with soil bacteriology.

Fermentation of Pectin, the Process of Retting.— Pectin is a gumlike compound closely related to the carbohydrates, occurring generally in plant tissues. In constitutes the major portion of the binding material which goes to make up the structure known as the middle lamella. A careful study of a cross-section of any plant tissue will

show that each individiual cell has its own separate cell wall made up of cellulose, and that there is a binding material between the two cellulose walls holding the cells together in the form of a tissue. In some plants this middle lamella is relatively thick. In other cases it is thin and can be demonstrated only with some difficulty. Microörganisms of several kinds are known which are capable of producing the enzyme pectinase which softens and dissolves this pectin, thus allowing cells or groups of cells to fall apart.

Bacteria capable of producing pectinase are of considerable importance in certain plant diseases. The disease soft rot or black rot of cabbage may be used as an illustration. In this disease the bacteria gain entrance to the tissues of the leaf through the water pores or through insect injuries. They begin to grow in the intracellular spaces, producing the enzyme pectinase which diffuses between the cell walls, softens and removes the pectin, and the cells no longer are joined together into a tissue. Microscopic examination of rotting or rotten wood, for example, will show that the cells are no longer joined together and they may be readily separated from each other.

A practical utilization of this property of pectinase formation by microörganisms is to be observed in the process known as retting. The long cellulose bast fibers of the flax plant and of the hemp plant are used largely in textile industries in the manufacture of linen and hemp articles. In the mature plants the bundles of bast fibers are so closely united to other plant tissues that it is not practicable to remove them and clean them by mechanical means. In order to soften the middle lamella the plants are subjected to the process known as *retting*. It may be noted that this is the old English form of the word *rotting*. Literally the plants are rotted for a given length of time. The process of retting may be either anaërobic or aërobic in nature. In the former the bundles of flax or hemp are

bound and sunk in water of a pond or stream. Certain anaerobic bacteria closely related to the clostridia already noted as bringing about cellulose fermentation find conditions favorable for growth under these conditions and produce pectinase, dissolving and digesting the binding elements between the bundles of bast fibers. Several species have been described as instrumental in bringing about this change. The ones most commonly noted are *Clostridium asterosporum* and *Clostridium pectinovorum*. When the

FIG. 53.—CLOSTRIDIUMS. 1. Clostridium butyricum. 2. Clostridium pectinovorum.

process has continued sufficiently long and before these microörganisms attack the cellulose, the bundles are removed, dried and are ready for the extraction of the bast fibers which can now be readily accomplished by mechanical means. In the aërobic process the flax or hemp is allowed to lie on the surface of the ground exposed to the dew and rain. Under these conditions certain molds and possibly certain bacteria as well, grow through the stems and gradually bring about the same kind of change as in water retting. It may be noted that pure cultures of anaërobic retting bacteria have been prepared and their use has proved successful in an experimental way.

FERMENTATION OF STARCH AND INULIN

Starch and inulin are two of the most commonly formed polysaccharides stored as reserve food material by plants.

Starch appears in plant tissues and plant cells in the form of starch grains. These are insoluble in cold water. The starch has the empirical formula $(C_6H_{10}O_5)_n$. Inulin on the other hand is soluble in cold water, does not form granules in plant tissues, but is widely distributed in the great family of plants known as the composites. It is abundant, for example, in the sunflower, the dahlia, the dandelion and chicory. It has the same empirical formula as starch. Upon hydrolysis starch breaks down through a series of dextrins to maltose and finally to dextrose. Inulin, on the other hand, breaks down through a series of intermediate compounds to fructose (levulose).

Starch and inulin may be fermented in two principal ways. Some microörganisms hydrolyze the complex carbohydrates into simpler compounds, finally forming sugar. They may or may not utilize the sugar thus formed, though usually they do. Other microörganisms break down starch but utilize the intermediate products, changing them into acids, gases, acetone and other compounds. In this process sugar apparently is never formed or at least, if formed, is so evanescent that its presence is not detected.

Microörganisms capable of bringing about hydrolysis of starch are of considerable economic importance. It has already been noted that certain fungi may be utilized commercially in the transformation of starch into sugar preliminary to the manufacture of alcohol in the so-called *amylomyces* process. Apparently the first utilization of fungi for saccharification of starch was in oriental countries, probably in China. The Chinese use this method in the preparation of a medium for the growth of yeasts in the manufacture of Chinese whisky. Rice is first softened by boiling, mixed with chopped rice straw, and made into cakes. These cakes are exposed to the air and allowed to mold. The molds most frequently occurring are certain species of *Aspergillus,* particularly *Aspergillus oryzæ,* and several species of *Mucor* and *Rhizopus.* When the mold is

well developed the cakes are dried and kept for future use. In the manufacture of the rice whisky, rice is boiled until soft, cooled, and inoculated with these moldy rice cakes. The spores are thoroughly stirred into the mixture. They soon germinate and the mycelium produced develops abundance of amylase. The starch is rapidly converted by this means into sugar and the alcoholic fermentation resulting from yeast introduction may go forward.

Microörganisms capable of digesting or decomposing

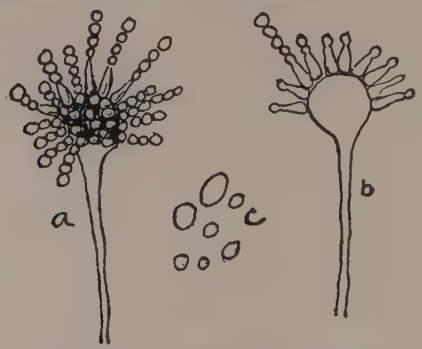

FIG. 54.—ASPERGILLUS ORYZAE.

starch and inulin without the formation of sugars are comparatively common. Many of the bacteria of the alimentary tract and of the soil can bring about these changes. Some of the products formed are the higher alcohols, such as amyl alcohol, and the various simpler acids of the fatty acid series, such as acetic, butyric and valerianic. The ability or lack of ability of various organisms to attack starch or inulin is of great importance in the differentiation of species in the bacteriological laboratory.

FERMENTATION OF FATTY ACIDS AND GLYCERIN

Fats are present in practically all plant and animal cells, sometimes occurring in considerable quantities. Fats and oils constitute a large proportion of the food of man and

animals, consequently changes brought about by microorganisms are of considerable economic importance.

Fats are glycerides of the fatty acids. Among the commoner fats are palmitin $(C_{15}H_{31}COO)_3C_3H_5$, and olein $(C_{17}H_{33}COO)_3C_3H_5$.

The fermentation of fats is usually primarily an hydrolysis of the fat into its constituent fatty acids and glycerin. This process may be illustrated by the following reaction:

$$\underset{\text{Stearin}}{(C_{17}H_{35}COO)_3C_3H_5} + 3H_2O = \underset{\text{Glycerin}}{C_3H_5(OH)_3} + 3\underset{\text{Stearin acid}}{C_{17}H_{35}COOH}$$

Microörganisms may then attack either the glycerin or the fatty acid, bringing about secondary fermentation.

The list of bacteria capable of bringing about fat hydrolysis is not very extensive. Many species of molds, however, are known which can bring about this change. It may readily be observed by growing the organism to be studied upon an agar plate, in which the agar contains an emulsion of the particular fat to be studied. The development of the characteristic enzyme, lipase, will give rise to a transparent area immediately surrounding the colony of the lipolytic organism.

Very few microörganisms are known which can directly digest or ferment fatty acids with long carbon chains. A considerable number of molds and bacteria, however, are able to utilize the volatile acids of the fatty acid series. It has already been noted that molds and bacteria may oxidize acetic acid, butyric acid and their salts.

Many species of bacteria are capable of fermenting glycerin. This may be regarded as a simple carbohydrate. It is one of the substances frequently used in the laboratory for differentiating the fermentative powers of microörganisms and for consequent characterization of species.

CHAPTER XIX

DECOMPOSITION OF ORGANIC NITROGENOUS COMPOUNDS —RIPENING OF CHEESE AND OF MEAT

All plant and animal tissues and cells contain proteins, and frequently other complex nitrogenous compounds. They probably are the most essential of the constituents of protoplasm. They contain the elements carbon, hydrogen, oxygen, nitrogen, frequently sulphur or phosphorus, and sometimes iron. They may be characterized chemically as exceedingly complex nitrogenous compounds, which, when hydrolyzed, break down into a large proportion of alpha-amino acids. Conversely it may be stated that proteins are built up by combinations of various amino acids.

The general formula for the alpha-amino acids is $RCH{\cdot}NH_2{\cdot}COOH$. Some nineteen or twenty distinct alpha-amino acids have been found by chemists in their studies of the hydrolysis of protein, that is to say, the R in the generalized formula may represent some nineteen or twenty different groupings. The manner in which the proteins are built up may be illustrated by the use of glycocoll, one of the simplest of the amino acids, as an example. Glycocoll is an amino-acetic acid, CH_2NH_2COOH. It has been found possible by chemical means to cause two molecules of an alpha-amino acid to unite with each other. It may be noted that these acids contain both an acid (carboxyl or COOH) and a basic (NH_2) group. The NH_2 (or basic portion) of the molecule may be caused to combine with the carboxyl radical of another. Disregarding the intervening steps

necessary in bringing about this combination, the reaction may be written:

$$CH_2NH_2COOH + CH_2NH_2COOH = NH_2CH_2CONHCH_2COOH + H_2O$$

By the abstraction of a molecule of water, then, it is possible to cause alpha-amino acids to unite with each other. The resulting compound is called a *peptid.* An examination of the structure of the peptid shows it also to be an amino acid, that is, it contains an NH_2 and a carboxyl radical. It is, therefore, possible to duplicate the preceding reaction and cause peptids to unite with each other. If two dipeptids unite, for example, the resulting compound will be made up of four alpha-amino acid radicals joined together. This process may be continued indefinitely. As many as sixteen of these alpha-amino-acid radicals have been caused to join to each other in laboratory studies. A complex compound of this type is termed a *polypeptid.* In nature, undoubtedly, polypeptids unite to form *peptones,* peptones to form *proteoses* and proteoses finally to form *proteins.*

Microörganisms in bringing about changes may attack the proteins by adding water to the molecule and causing them to break down into simpler proteoses. The proteoses in turn may be hydrolyzed into peptones and these through polypeptids and peptids and finally to the alpha-amino acids. Such a change may be regarded as a digestive action on the part of the microörganisms. Such changes are due to the development of pepsin and trypsinlike ferments which act externally to the cell of the microörganism, that is, this is an example of the analytic action of an extracellular enzyme. The amino acids thus formed as the result of the activity of microörganisms may themselves be attacked in various ways. One of the most important of these is the process termed deaminization. Certain microorganisms have the power of removing the amino group,

leaving the corresponding acid behind. The amino group is thus converted into ammonia and the process from the standpoint of the soil bacteriologists is frequently termed *ammonification.* As an example of this change may be given the transformation of glycocoll into acetic acid and ammonia. The reaction may be written as follows:

$$\underset{\text{Glycocoll}}{CH_3NH_3COOH} + H_2 = \underset{\text{Acetic acid}}{CH_3COOH} + \underset{\text{Ammonia}}{NH_3}$$

It will be noted that this transformation requires the presence of nascent hydrogen. The change is one, therefore, which is essentially anaërobic. Microörganisms which grow in the presence of oxygen, that is, under aërobic conditions, may in some cases bring about deaminization by oxidation. In this case a fatty acid containing one less carbon atom in the chain is usually formed. The reaction may be indicated as follows:

$$\underset{\text{Alanin}}{CH_3CHNH_2COOH} + O_2 = \underset{\text{Acetic Acid}}{CH_2COOH} + NH_3 + CO_2$$

It is evident, therefore, that either under aërobic or anaerobic conditions microörganisms may free ammonia from amino acids. This is important because ammonia may be utilized directly, or after conversion into nitrates, by higher plants as nutrient material.

Certain microörganisms when growing under anaërobic conditions may attack amino acids eliminating carbon dioxide, thereby changing the compound from an amino acid to an amine. This process is termed *decarboxlyation.* It may be illustrated by the following reaction:

$$\underset{\text{Alanin}}{CH_3CHNH_2COOH} = \underset{\text{Ethylamine}}{CH_3CH_2NH_2} + CO_2$$

Amines produced as a result of the action of microörganisms are frequently termed *ptomaines.* Some of the amines

thus developed are malodorous. A few are distinctly poisonous. For example, the histamin formed by the decarboxylation of the amino acid called histidin is extremely poisonous. Among the ptomaines (amines) most commonly produced by microörganisms is trimethylamin $(CH_3)_3N$. This gives a characteristic odor to herring brine and decaying fish. It is produced in pure cultures by certain bacteria. It is not highly poisonous. Other ptomaines which have been isolated are putrescin (tetramethylenediamin, $NH_2C_4H_8NH_2$) and cadaverin (pentamethylenediamin, $NH_2C_5H_{10}NH_2$) and neurin $(CH_3)_3C_2H_3NOH$. The latter is very poisonous. It should be noted that apparently food poisoning is but rarely due to the presence of ptomaines. Most of the cases of ptomaine poisoning which have been recorded in the literature or which are popularly supposed to be of this type are in reality either infections with specific bacteria or are the result of eating foods containing true toxins or endotoxins produced by the specific bacteria, and are not primarily the result of a decomposed protein.

Certain microörganisms, particularly yeasts, change amino acids by hydrolysis to a primary alcohol, at the same time bringing about decarboxylation and deaminization. For example:

$$\underset{\text{Alanin}}{CH_3CHNH_2COOH} + H_2O = \underset{\text{Ethyl alcohol}}{CH_3CH_2OH} + NH_2 + CO_2$$

One of the common products in alcoholic fermentation is amyl alcohol or so-called fusel oil. This is produced by yeasts from isoleucin. The reaction may be illustrated as follows:

$$\underset{\text{Isoleucin}}{C_5H_{10}NH_2COOH} + H_2O = \underset{\text{Amyl alcohol}}{C_5H_{11}OH} + NH_3 + CO_2$$

The amyl alcohol thus formed finds extensive use in the industries, particularly in the form of amyl acetate.

Types of Organisms

A comparatively small number of species of bacteria are known which can attack pure protein in the absence of other nutrients. A much larger proportion are able to decompose proteoses, peptones and amino acids.

The microörganisms responsible for bringing about decomposition of protein may be grouped under the two headings, anaërobic and aërobic. The former produce most of the malodorous compounds ordinarily associated with putrefaction. The latter are responsible for decomposition in which such compounds are oxidized, that is, they bring about the change ordinarily termed decay.

The Anaërobic, Putrefactive Bacteria.—Certain members of this group of microörganisms are capable of causing disease. Such are the forms developing in blackleg and malignant edema in animals, and the gaseous gangrene in man. The saprophytic bacteria belonging to the group are comparatively numerous. The most important species is *Clostridium putrificum*. This organism is abundant in nature in the soil. Together with several closely related species it probably is the most common cause of putrefaction of meat and similar proteins. The organism itself is a slender rod, single or in chains. Its spores are developed in the ends of the rods, causing them to be swollen and assuming the shape of drumsticks. Microscopically the organism has much the same appearance as *Clostridium tetani*, the cause of tetanus. It grows readily in ordinary culture media. It is capable of developing in a mixture of egg white and water into which has been inoculated a little soil, cheese or fecal material. When growing under these conditions an intense putrefactive odor develops, probably due largely to the formation of hydrogen sulphide and possibly of methyl mercaptan (CH_3SH). Butyric acid and ammonia are also formed. This is one of the few bacteria

capable of bringing about decomposition of such native proteins as albumin or fibrin under anaërobic conditions without the presence of any carbohydrates. The bacteria may usually be seen readily in stained mounts taken from putrefying material.

The Aërobic, Proteolytic Bacteria.—Various bacteria belonging to the genera *Bacillus, Proteus* and *Pseudomonas,* together with certain molds are able to bring about decomposition of proteins under aërobic conditions. The organisms belonging to the genus *Bacillus* have usually been regarded as among the most important of the bacteria in the soil in bringing about decomposition of nitrogenous compounds. The recent work of Conn and others seems to throw some doubt upon their importance in the soil, inasmuch as apparently they are usually present in the form of spores. When grown in the laboratory, however, most species of the genus *Bacillus* are capable of digesting gelatin and frequently proteins such as albumin or egg white are digested as well. Typical of this group is the *Bacillus mycoides.* This organism is a comparatively large rod, usually occurring in long chains and, when grown upon culture media, producing rootlike extensions from the sides of the colonies. The spores are formed readily, are equatorial in position and do not cause an enlargement of the cell. It grows readily upon ordinary culture media. Milk, blood serum and gelatin are all liquefied. The organism is particularly active in the development of ammonia.

Members of the genus *Pseudomonas,* such as *Pseudomonas fluorescens,* and members of the genus *Proteus,* such as *Proteus vulgaris,* are probably also important in bringing about ammonification.

Certain molds, particularly members of the genus *Penicillium,* are active in producing hydrolysis in certain proteins, particularly the proteins of milk. It will be noted

later that they are active in bringing about the changes incident to the ripening of certain kinds of cheese.

Proteolytic Changes in Foods

The Ripening of Cheese.—Cheese is a product prepared from milk by causing a coagulation or curdling of the simple milk protein and a more or less complete removal of the liquid portion or whey. Many different kinds of cheeses are prepared. Probably in all more than one hundred different varieties have been described. There are several factors which contribute to determine the character of a cheese. They may be enumerated as follows:

1. *The kind of milk.*—Cheese is prepared in various countries from the milk of the cow, goat, sheep and mare. These various milks differ somewhat in chemical composition and consequently cause variation in the composition of the cheese produced.

2. *The method of causing curdling.*—Milk may be curdled either by the development or addition of acid or by the addition of the enzyme rennet. The curds produced by these two methods are not identical.

3. *The proportion of whey expelled.*—The amount of moisture left in the cheese is determined by the amount or proportion of the whey squeezed out. Cheeses are sometimes classified as moist and dry.

4. *The size of the cheese prepared.*—Small cheeses give opportunity for microörganisms growing upon the surface in the presence of oxygen to exert an influence on the character and flavor of the cheese. This does not occur in the large cheeses.

5. *Amount of salt and condiment.*—Various materials are used to flavor cheeses.

6. *The temperature of ripening.*—The temperature at which the cheese is held during the ripening process may

make a marked difference in the texture and flavor of the product.

7. *Time of ripening.*—Some cheeses require a very much longer period of time for their complete ripening than do others.

8. *The organisms present and active in the ripening process.*—The kinds of microörganisms present and active will depend very largely upon the factors already enumerated. Perhaps it may be emphasized that all the preceding conditions are attempts to vary the conditions under which microörganisms will be active and consequently vary the type and proportion of products developed. In some cases particular microörganisms will be active and consequently vary the type and proportion of products developed. In other cases, certain microörganisms are intentionally introduced. Usually they are present in the material and there is no need of special inoculation.

Types of Cheeses.—Cheeses may be differentiated into acid curd cheeses and rennet curd cheeses; the latter, in turn, into the hard cheeses and soft cheeses.

Acid Curd Cheeses.—Acid curd cheeses are prepared by allowing the milk, usually whole milk, to sour spontaneously or after the introduction of a suitable starter. When the acidity has reached a suitable point the material is heated, whereupon the whey separates out. The curd is then made into balls or cakes and sometimes mixed with cream or butter before use. The so-called Dutch cheeses and cottage cheeses prepared in the household are of this type. They are usually not ripened before they are used. By a special process, sour curd cheeses are ripened in some of the Scandanavian countries and a peculiar type of cheese produced. Ordinarily acid curd cheeses are not held for very long periods of time, inasmuch as the yeasts and molds soon make the cheese unfit for use.

Rennet Curd Cheeses.—Of the many types of rennet curd

cheeses produced, three only may be mentioned as illustrating various processes of ripening and of manufacture. These are Cheddar cheese, an example of a hard cheese, and Camembert and Roquefort, examples of soft cheeses.

Ordinary Cheddar cheese is prepared from milk of good quality, care being used that gas-producing bacteria of the *Bacterium aërogenes* or *Bacterium coli* type are not present in sufficient numbers to cause gas bubbles to develop in the ripening cheese. The milk is allowed to stand until a small amount of acid, usually about .2 per cent has been developed. The rennet is then added, whereupon the milk quickly curdles. The curd is broken up by means of knives and gradually shrinks until most of the whey is drained off. The whey is finally removed quite completely by pressing and the curd is formed into large cakes. The consistency of the casein gradually changes until it becomes rubbery and unites readily to form a coherent mass. The cheese is then placed in the curing room until the ripening process has been completed. The ripening is probably due to the combined action of proteolytic enzymes originally present in the milk and introduced with the rennet, and the proteolytic and fermentative action of certain bacteria which are present. Most of the lactose is soon transformed into lactic acid. This acid in large part combines with the casein and furnishes suitable conditions for activity of the pepsinizing or proteolytic enzymes. The proteins are attacked and partially hydrolyzed, that is, the casein becomes more and more soluble in water. Flavoring substances of various types are produced. It is probable that these are in part ammonia and related compounds, in part volatile acids, and in part the ethyl esters of various fatty acids.

Roquefort cheese was originally made from sheep's milk but milk of the cow is also used to some extent. This is a characteristic soft cheese in which opportunity has been afforded for the growth of a specific mold, *Pencillium*

roquefortii, throughout the mass. Air is supplied by means of holes punched through the cheese by stiff wires. The cheese is originally inoculated in some cases by the addition of moldy bread crumbs to the curd.

Camembert cheese is also a soft cheese in which a much larger proportion of whey is retained than in Cheddar cheese. The cheese is molded into a small mass and kept in a suitable curing room. Certain molds soon cover the entire surface of the cheese. The most important of these are the *Oöspora lactis* and various species of *Pencillium*, particularly *Pencillium camembertii*. These molds, particularly the latter, produce a proteolytic enzyme which gradually diffuses toward the interior of the cheese. There is, therefore, a progressive softening of the cheese mass from the exterior to the interior, and the cheese is considered completely ripe when the softening has extended to the center. The mold itself usually does not penetrate very deeply into the cheese mass and is removed before the cheese is consumed.

Ripening of Meat.—The so-called ripening process occurring in meat is not brought about by the activity of microorganisms, although there are certain types of sausages in which some bacterial and mold action is important in contributing to the flavor. The ripening of meat is for the most part due to the activity of the autolytic proteolytic enzymes present in the meat itself.

CHAPTER XX

BACTERIA OF THE SOIL—THE CYCLES OF THE ELEMENTS

Importance of Bacteria in the Soil.—Were it not for the presence and activities of the bacteria in the soil the growth of higher plants would soon become impossible. By their activity the soil bacteria are constantly making available for the higher plants the various substances necessary for their growth. Growing crops need, among other elements, carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, iron and calcium. Bacteria play only a minor rôle in making available the carbon, hydrogen and oxygen, but the other elements are practically all of them made available in suitable compounds by the action of the bacteria.

Abundance and Distribution of Bacteria in the Soil.—No entirely satisfactory method has been worked out for determining the actual numbers of bacteria of all kinds in a sample of soil. By plating and dilution methods, it is possible to secure approximate counts of many kinds of microörganisms, but some forms do not grow readily upon ordinary culture media and may be overgrown or lost entirely. The actual numbers which may be enumerated vary from a few thousand to many million per gram of soil. Of the microörganisms present the most abundant usually are bacteria, next come molds, yeasts and the protozoa. The species which are present, particularly in their comparative proportion of individuals, differ greatly, depending upon climatic conditions and the character of the soil. They are most abundant near the surface, in general, and decrease rapidly with increase in depth. In some soils which are

uniform to a great depth, as in certain of the semiarid soils of the western United States, bacteria are moderately abundant to considerable depths.

Cycles of the Elements.—Bacteria, yeasts and molds in the soil are of primary importance because of the chemical changes which they are able to bring about. The changes which have been enumerated above make available elements necessary for plant nutrition. Complex compounds are constantly being broken down into simpler compounds and simple compounds built up again into more complex forms. These successive transformations and changes in complexity of the various important elements may be usually grouped together under the heading of the term "cycle." The elements which are important from this point of view are nitrogen, sulphur, phosphorus, iron, carbon and calcium.

The Cycle of Nitrogen in Nature with Special Reference to the Soil

Outline.—The transformations which nitrogen may undergo are relatively numerous. Microörganisms play a large part in bringing about these changes. Simple nitrogen compounds are assimilated by higher plants and built into complex compounds. These, after the death of the plant, or of the animal which has eaten the plant, are returned to the soil and broken down into simpler compounds. As the starting point in the discussion of the cycle of nitrogen one may take the more complex protein. Many microörganisms may attack this, bringing about the change termed *proteolysis*. Eventually the nitrogen appears in the form of ammonia and the process termed *ammonification* is complete. Various microörganisms in the soil are capable of oxidizing ammonia-producing nitrities. Still other bacteria oxidize the nitrites to nitrates. These are assimilated by the green plant, gradually built up into compounds more and more complex until finally plant proteins are de-

veloped. Under certain conditions microörganisms may attack nitrates, removing oxygen and changing them to nitrites. Nitrites may also be attacked or reduced and free nitrogen given off. These processes are usually termed *denitrification*. Certain microörganisms are likewise capable of taking up atmospheric nitrogen and converting it to their own use, or in some cases turning it over to higher plants, particularly the legumes. This process is termed *nitrogen fixation*. For convenience in discussion it is

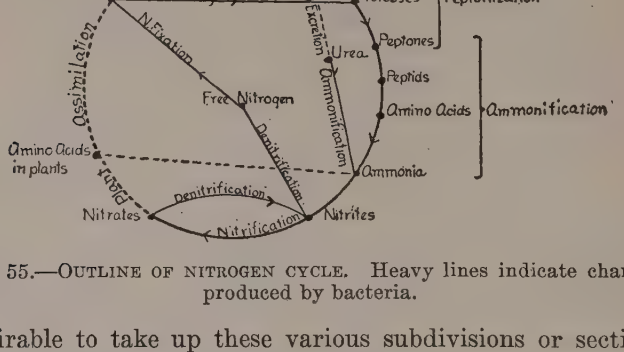

FIG. 55.—OUTLINE OF NITROGEN CYCLE. Heavy lines indicate changes produced by bacteria.

desirable to take up these various subdivisions or sections of the nitrogen cycle one after the other.

Proteolysis, Ammonification or Deaminization.—The most complex of the nitrogen compounds are the proteins. They have already been defined as complex organic compounds made up of carbon, hydrogen, oxygen and nitrogen, sometimes including also phosphorus, iron or sulphur, and which yield, upon hydrolysis, a large portion of alpha-amino acid ($RCHNH_2COOH$). It has also been shown that it is possible for the chemist to join chains of amino acid radicles indefinitely, the cement which joins them together being the *abstraction of water*. When proteins are decomposed as the result of the action of digestive enzymes or the

action of bacteria, a process the converse of that just discussed occurs. Water is inserted into the complex molecule which then breaks apart at this point of cleavage forming two molecules somewhat less complex. Bacteria which are capable of attacking proteins and breaking them down in this fashion by the introduction of water, that is, by hydrolysis, are said to be *proteolytic*. Such organisms are comparatively common in the soil. Many of the spore-producing bacteria, such as *Bacillus subtilis* and *Bacillus mycoides* are of this character. The same kind of a change may be brought about by some of the species of cocci and by species of *Actinomyces*. The proteoses and peptones produced by these organisms are attacked by a still greater variety of forms and broken down into simpler and simpler compounds, eventually with the formation of considerable amounts of alpha-amino acids. These amino acids, as they develop, may be attacked by species of bacteria and molds in the soil. One of the most common changes is that known as *ammonification*, or more properly *deaminization*. Ammonia is split off. In most instances apparently the microörganism is not primarily concerned with the ammonia which is thus released, but has removed the amino group in order that it may utilize the remainder of the acid as food.

It is evident that not all of the nitrogen compounds which gain access to the soil are in the form of plant and animal protein. A considerable part, for example, of the nitrogen in barnyard manure may be in the form of urea and hippuric acid. Many species of spore-producing bacteria in particular are capable of attacking urea, bringing about deaminization or ammonification in accordance with the following reaction:

$$(NH_2)_2CO + 2H_2O = (NH_4)_2CO_3$$

Other nitrogenous compounds which may be added to the soil such as hippuric acid, calcium cyanamide, etc., are

deaminized in an analogous fashion. In general, it may be noted that practically any organic compound of nitrogen is eventually broken down by soil microörganisms with the formation of ammonia.

Well-aërated soils show little tendency for ammonia to accumulate. As rapidly as it is formed it is changed by the nitrifying bacteria, but under anaërobic conditions, such as exist in the mud in the bottom of a pond or in water-logged soil, the nitrogen remains more or less indefinitely in the form of ammonia. Not infrequently the fact that the ammonia is usually oxidized in arable soils and the nitrogen available to the roots of green plants, therefore, is generally nitrate, has led to the erroneous assumption that green plants cannot take up ammonia through their roots. In general, this is a fallacy. In fact, many kinds of plants, particularly those which live with their roots in wet or water-logged soils or in mud, secure practically all of their nitrogen in the form of ammonia. However, ammonia is relatively volatile and would be readily lost from the soil and manure were it not for the fact that it is, in the presence of oxygen, readily oxidized to nitrites and nitrates.

Inasmuch as the rapidity with which the microörganisms in a given soil can bring about complete proteolysis and ammonification of nitrogenous manures added is one of the indices to soil fertility, methods have been devised for testing out this soil power in the laboratory. The test is carried out by adding a given amount of a particular nitrogenous material, such as casein, blood meal or other organic nitrogenous compound, to a given weight of the soil to be tested, mixing thoroughly, placing in suitable vessels, usually tumblers, adding water to the optimum and incubating. At the end of a given period of time these samples of soil together with similar samples to which no manure has been added are analyzed for their ammonia content.

Until recent years it has generally been thought that the

spore-producing bacilli in the soil were the most important of the ammonifying bacteria. It is undoubtedly true that in the laboratory in culture media these microörganisms are capable of digesting proteins quite readily. Careful studies of soils, however, seem to indicate that most of these species are present except under unusually favorable conditions, in the form of spores and that they are numerous in the soil not so much because they are rapidly multiplying or are present in the vegetative stage, but because there is a gradual accumulation of highly resistant spores. The bacteria apparently, which are somewhat more important in bringing about ammonification, are members of the genus *Pseudomonas,* particularly forms of the type *Pseudomonas fluorescens.* These bacteria can likewise bring about rapid ammonification in laboratory media.

It is probably a very unusual condition for soils to be markedly deficient in microörganisms capable of bringing about ammonification. The bacteria are probably most important in ordinary arable soil, although in some acid soils, in leaf mold and in similar locations, it is probable that molds are more important.

Though the microörganisms in the soil usually are producing ammonia from complex nitrogenous compounds greatly in excess of their own needs, nevertheless, it must be remembered that some of the ammonia is assimilated by them. That is, some of the nitrogen of the soil becomes locked up in the cells of the bacteria, yeasts and molds present. Chemical analysis of the soil, therefore, will always show a small portion at least of the nitrogen in the soil in organic form. This may later be released by the death and decomposition of the bacterial or mold cells.

Nitrification.—The term "nitrification" as applied to the transformation of ammonia into nitrites and finally into nitrates is a misnomer chemically. The process is actually one of oxidation. The name, however, has become so firmly

fixed in discussions of soil bacteriology that it probably cannot be replaced by a better one.

The process of nitrification as it occurs in soils usually takes place in two distinct steps; first, the oxidation of ammonia to nitrous acid or nitrites, a process which may be termed *nitrosation,* and second, an oxidation of the nitrous acid or nitrites to nitrates, a process termed *nitratation.*

The process of nitrosation is brought about by a peculiar group of prototrophic bacteria. All of them belong to the genus *Nitrosomonas.* Some are short rods and motile. Others are spherical and nonmotile. They do not grow at

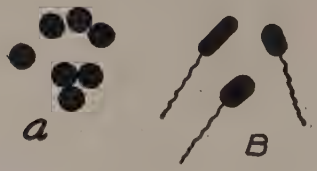

FIG. 56.—NITROSOMONAS. A. Nonmotile spherical series. B. Motile rod-shaped form.

all readily in the laboratory upon the commonly used media and may be isolated and studied only by the use of special methods. They require for their growth the presence of ammonia and free atmospheric oxygen. For the most part they do not grow well in the presence of an abundance of organic matter, particularly of amino acids and peptone. They are found in the laboratory not to require any organic matter whatever for their development. Apparently they oxidize the ammonia in accordance with the following equation:

$$2NH_3 + 3O_2 = 2HNO_2 + 2H_2O$$

and secure by this means energy for their growth and for the manufacture of food. They are capable of securing

carbon from carbonates and building this up into complex compounds.

The organisms belonging to this genus of bacteria may be isolated in the laboratory by placing a small amount of soil in a solution containing ammonium sulphate, carbonates and salts (not nitrogenous), such as phosphates, necessary for the growth of bacteria. A clouding of the medium will eventually develop and a microscopic examination show the characteristic bacteria to be present in abundance. Chemical tests will also show that a portion, if not all, of the ammonia has been transformed into nitrite. It may be emphasized that organisms such as these must be among the most primitive of living things. They are capable of living wholly upon inorganic sources of materials and they do not even require sunlight for securing energy for manufacture of their food. They may be grown in pure culture in media such as agar or silica jelly containing ammonium sulphate and suitable salts.

It may further be emphasized that these microörganisms do not oxidize the ammonia to nitrite in order to make use of the nitrite formed, but rather that they may secure energy from the oxidation. The nitrites then constitute a waste product of the bacterial metabolism. Very rarely is there any tendency for the nitrites to accumulate in appreciable quantities in any soil. The conditions which favor the growth of the *Nitrosomonas* will also favor the growth of organisms which can transform the nitrites to nitrates. Whenever nitrites are present in abundance in soils, it may be taken as evidence that they have formed, not as a result of the oxidation of ammonia, but as a result of anaërobic denitrification, to be discussed later. Nitrites are not utilized by higher plants as a source of nitrogen. In fact, they are in general injurious to plants, particularly to plant roots.

Nitrites as rapidly as they form in soil are usually

oxidized into nitrates by bacteria belonging to the genus *Nitrobacter*. These are small, nonmotile rods present in most arable and fertile soils. Like *Nitrosomonas* they do not require the presence of organic material for their development. They are likewise prototrophic and secure their growth energy by the oxidation of the nitrites to nitrates in accordance with the following reaction:

$$2HNO_2 + O_2 = 2HNO_3$$

Nitrates, therefore, tend to accumulate in the soil. Under certain conditions they may be leached out and concentrated in other places. It is claimed, for example, that the great nitrate deposits of certain arid regions, as in Chile, are due to the concentration brought about in this way. Nitrates are easily taken up by the roots of most higher plants. Consequently it is in this form that nitrogen is assimilated by most growing crops.

Nitrobacter may be readily cultivated in the laboratory by preparing a solution containing potassium nitrite, carbonates, phosphates and other salts necessary for growth, but containing no organic material. When such a solution is inoculated with a bit of soil a clouding will soon develop and the nitrites will be found to be changed to nitrates. The oxidation may be materially increased by suitable aëration. The ability of a particular soil to transform nitrites into nitrates may be tested by a manner similar to that already noted for ammonification, but in general it is not differentiated from the process of nitrification as a whole.

It has already been noted that the process of nitrification, or oxidation of ammonia, starts with the ammonia and ends with nitrates. The ability of soil to bring about this entire change of nitrification may be studied by adding ammonium sulphate or some other compound containing available ammonia to a suitable soil sample in the laboratory, adding the optimum amount of water and analyzing after a suit-

able period for the content in nitrates. Determination of the ammonifying and nitrifying power of soils is among the more valuable determinations made in the laboratory in an effort to estimate soil fertility.

Assimilation of Nitrogen by Plants.—All plants which live in the soil, the bacteria, the yeasts, the molds, the higher fungi, many algæ, the mosses, the ferns, and the seed plants all require nitrogen for their growth. The process of taking up nitrogen and utilizing it in metabolism, particularly in the building up of protoplasm, is termed *nitrogen assimilation.*

It was previously noted that bacteria of different kinds utilize many different sources for their nitrogen supply. A few species are capable of taking it directly from the air. Many more can utilize amino acids, ammonia or nitrates. Nitrogen taken up by bacteria and built into protoplasm is locked up, of course, in a form unavailable to higher plants. Molds and yeasts resemble bacteria in their ability to use various compounds of nitrogen in their metabolism. It is not strange, therefore, that in fertile soils an appreciable amount of the total nitrogen contained in the soil should be found in the cells of the yeasts, molds, bacteria and other fungi present.

Some higher plants have the ability to assimilate amino acids, but these latter are present usually in very small quantities and only at intervals in most soils. Many species likewise can utilize ammonia, and most species can utilize nitrates. Nitrites in general are poisonous to plant roots. The nitrogen upon entering the plant is rapidly built up into more complex nitrogenous compounds; first, probably into amino acids, then into peptids, then finally into the complex plant proteins. Inasmuch as animal proteins are in all cases derived directly or indirectly from plant proteins this may be said to complete the nitrogen cycle. There are, however, several transformations of nitrogen not in-

cluded in the main cycle which are of great importance from an economic point of view. These are *denitrification* and *nitrogen fixation.*

Denitrification.—Denitrification is a term applied to the reduction of nitrates to nitrites and to the process of evolution of free atmospheric nitrogen from either nitrates or nitrites by the activity of microörganisms. Like the term nitrification it is a misnomer, as the process is essentially a reduction.

Nitrates are transformed to nitrites by certain kinds of bacteria when placed under anaërobic conditions and in the presence of certain easily oxidizable substances. For example, in the presence of organic material and the absence of free oxygen, certain microörganisms remove oxygen from the nitrate molecule, reducing it to nitrite, and use the oxygen thus obtained for oxidizing carbon compounds. A reaction of this type may be illustrated as follows:

$$C_6H_{12}O_6 + 12HNO_3 = 6CO_2 + 6H_2O + 12HNO_2$$

As a result of this action energy is secured for growth. The reduction of the nitrate requires less energy than is secured by the oxidation of the carbon compounds, hence there is a net gain in energy to the microörganism.

Many bacteria are known which bring about this transformation. Most of them are termed *facultative,* as they will grow well under normal aërobic conditions, but do not grow in the absence of atmospheric oxygen unless an adequate supply of this element can be secured from sources such as nitrates. The reduction of nitrates to nitrites is often observed in the laboratory and is a valuable means of differentiation of bacteria from each other.

Certain bacteria, the true denitrifiers, are capable of bringing about a reduction of nitrites with the evolution of free nitrogen gas. The chemistry of this transformation

has not been adequately worked out. The reaction itself, however, may be illustrated as follows:

$$C_6H_{12}O_6 + 8HNO_2 = 6CO_2 + 10H_2O + 4N_2$$

It is probable that in many cases the evolution of elementary nitrogen is due to the simultaneous presence of nitrites and amino acid. In general an amino acid and nitrous acid react with each as follows:

$$\begin{array}{l} R \cdot CO \cdot COOH + HNO_2 = R \cdot CHOH \cdot COOH + H_2O + N_2 \\ \quad | \\ \quad NH_2 \end{array}$$

To what extent denitrification can occur in the absence of amino compounds is not well understood. This latter reaction explains the small amount of nitrogen gas frequently found accompanying carbon dioxide or hydrogen in fermentation tests of bacteria of the colon typhoid group. Many of these forms apparently can transform nitrates into nitrites but cannot directly attack the nitrite molecule. If amino acids are present, however, there may be an evolution of nitrogen gas.

Denitrification is negligible in its results in most well-aërated soils. It may prove to be of importance, however, in certain soils containing considerable quantities of nitrate which have become water-logged. The free oxygen soon disappears and the nitrate may be transformed into nitrite by the bacteria present. It is possible that in some cases the amount of nitrites formed in this fashion may be sufficient to exert a toxic action upon plant roots. It has been found, for example, that the addition of nitrate as a fertilizer to rice is not good practice but that ammonia salts are much to be preferred. Apparently, this is because the roots of the rice grow in a water-logged soil containing little free oxygen. Nitrates may be reduced to nitrites and rendered not only unavailable to the plant roots, but actually toxic. Ammonia salts, on the other hand, are not trans-

formed and may be taken up and utilized directly by the plant.

Nitrogen Fixation.—A study of the cycle of nitrogen makes it evident that the amounts of living material upon the earth, that is, the number of plants and animals, must depend quite directly upon the amount of nitrogen in the nitrogen cycle. There are several ways in which nitrogen may be lost. First, there is the occasional process of denitrification and evolution of free nitrogen gas. Then explosives give off free nitrogen when fired. Fuel, when consumed, gives off some free nitrogen. It is evident that unless there is some way in which this nitrogen can be replaced in compounds, the number of living creatures on earth must gradually diminish. Until methods of fixation of atmospheric nitrogen were discovered this fact was a matter of grave concern to chemists. Laboratory studies have shown that nitrogen may be fixed, that is, atmospheric nitrogen may be combined with the other elements, by the use of high temperatures or the electric current. It is noted that there is some such combination of nitrogen during the electric discharges of a thunder storm. The amount of nitrogen fixed in this way, however, is very small in comparison to the amount fixed by the activity of microörganisms, particularly of bacteria and perhaps of some of the molds.

Nitrogen as an element is exceedingly inert. The nitrogen molecule is apparently in a stable condition and in order to force it to combine with other elements there is usually necessary an expenditure of energy. This is evident in the laboratory, for, as has been stated, high temperatures or the electric current must be utilized. These forces are not available to microörganisms. It is necessary, therefore, in all cases that some easily oxidizable food material be present so that energy may be secured for fixation. In most cases the nitrogen-fixing bacteria utilize carbohydrates.

The microörganisms capable of fixing atmospheric nitrogen are in part symbiotic, that is, living upon or in the roots of higher plants, and nonsymbiotic, that is, living free in the soil. Under each of these headings bacteria and fungi are to be considered.

Nitrogen Fixation by Symbiotic Bacteria.—The bacteria which live in the roots of plants and are capable of fixing atmospheric nitrogen belong to the genus *Rhizobium.* Whether or not more than one species should be recognized is somewhat uncertain. There is some evidence that there

FIG. 57.—RHIZOBIUM LEGUMINOSARUM. A. Peritrichous type. B. Monotrichous type. 1. Motile rods. 2. Non-motile or involution forms.

are several distinct species on the roots of distantly related leguminous plants. For the present discussion, however, all may be regarded as belonging to a single species termed *Rhizobium leguminosarum.*[1]

This organism is apparently widely distributed in soil. Whether or not it multiplies actively in soil is not very definitely proved. It is usually found associated with the roots of leguminous plants, causing the formation of nodules, sometimes termed "tubercles," upon them. No species of leguminous plant is known which, under its native conditions, does not have these nodules appearing upon the

[1] This organism is variously known in the literature as *Bacillus radicicola* and *Pseudomonas radicicola.*

roots. The family *Leguminosæ* is a relatively large one. Some forms are trees, some are shrubs, and many are herbaceous. They are found in abundance from the tropics to the arctic region, and from the low plain to the limits of vegetation upon the mountain. Some species develop best in very moist soil, others under relatively arid conditions. Some species prefer soils which are distinctly acid. Most species prefer a neutral to a somewhat alkaline soil. It is evident, therefore, that the *Rhizobium leguminosarum* is practically world-wide in its distribution. Apparently the only conditions under which it does not grow are those under which legumes will not grow.

Rhizobium leguminosarum may be readily cultivated in the laboratory from the nodules of legumes. It grows best on neutral media containing suitable sugars and phosphate. Peptone is not necessary. The colonies which develop upon sugar agar are usually somewhat slimy, sometimes almost clear and colorless. In sugar broth the medium becomes clouded and with some strains considerable amounts of gum are produced. This may be precipitated by the addition of alcohol. The bacteria grow readily upon a nitrogen-free medium and are able to fix appreciable quantities of atmospheric nitrogen. In determining the relative nitrogen-fixing power of various types of these bacteria, it is customary to compare the number of milligrams of atmospheric nitrogen fixed per gram of sugar (usually glucose) used. Usually *Rhizobium leguminosarum* is able to fix from one to five milligrams of nitrogen per gram of glucose utilized. The organism is aërobic, completely oxidizing sugar with the development of carbon dioxide and water. There is, therefore, an abundant evolution of gas, but as the oxidation can take place only near the surface of the medium and in the presence of an abundance of oxygen, gas bubbles are not observed and no gas is formed in the closed arm of the fermentation tube.

The young bacteria are motile, Gram-negative rods. There is some question as to the distribution of flagella. Some authors have claimed that a single polar flagellum is to be demonstrated, others that there are several flagella distributed over the surface of the cell. This may be due to the fact that in reality several species of bacteria have been studied, or because the cultures examined were of different ages. In many media the older organisms become considerably enlarged and more or less vacuolate. Frequently they are decidedly irregular. Branched forms and forms showing irregularities in staining are common. The appearance frequently leads to the inference that multiplication is by a process of budding and not by normal transverse fission.

While there are some points not well understood, in general the method of root infection has been fairly well worked out. The bacteria in the soil apparently come in contact with the root hairs. It will be recalled that root hairs develop just back of the root caps of the young growing roots and are somewhat evanescent. Older roots do not show them. In some way this species of organism finds a way to penetrate the wall of the root hair. Apparently it digests its way through. Once inside the root hair it begins multiplying relatively rapidly and produces a gelatinous strand which was formerly regarded as a fungous filament. In fact, this organism was originally described as a root-dwelling fungus. The strand of bacteria increases in length until it comes in contact with the cell wall toward the interior of the root. Here a buttonlike enlargement in the strand may take place. Eventually the bacteria make their way through the cell wall and invade the next cell. Infection of the root has now been established.

The presence of the organisms in the cells of the roots leads to an increased growth. It is probable that some substance excreted by the bacteria possesses the property

of stimulating the meristematic cells to increased rapidity of development. In consequence a small tumor or mass of

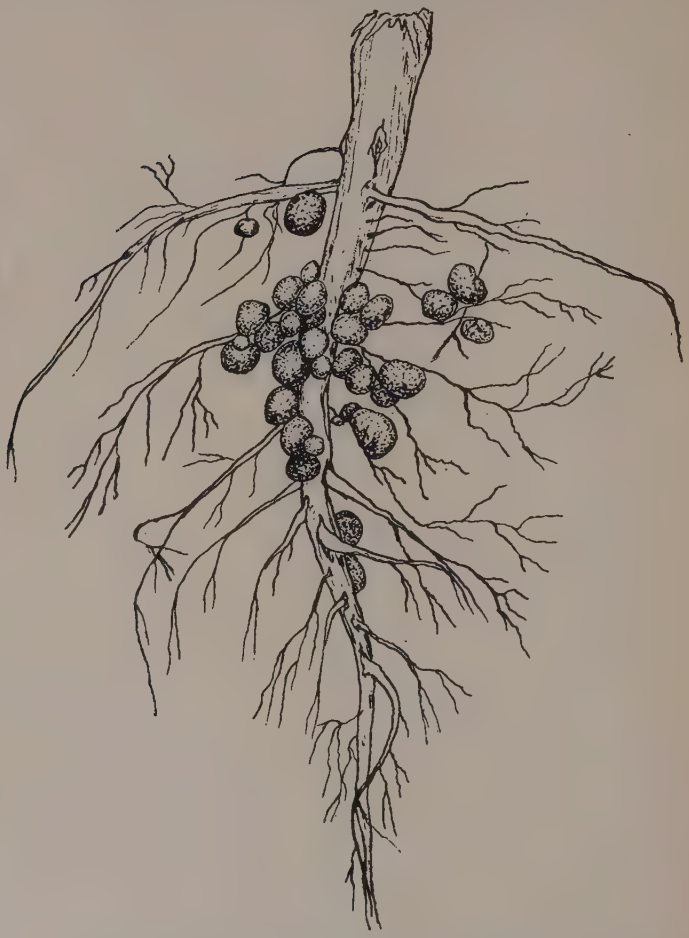

FIG. 58.—NODULES ON ROOTS OF A LEGUMINOUS PLANT.

cells develops rapidly and protrudes from the main portion of the root as a nodule. This nodule may be regarded as a modified rootlet.

The nodules of different legumes show some differences in structure. In general, however, there is an epidermal layer with a portion of the interior consisting of large cells more or less compactly filled with bacteria. Scattered among these cells there may be uninfected cells, in some cases packed with starch granules. Fibrovascular bundles leave the main root and pass from base to tip of the nodule

FIG. 59.—SECTION OF NODULE OF ROOT OF LEGUMINOUS PLANT. Cells containing bacteria shaded black.

just underneath the epidermis. It is interesting to note that in these bundles the arrangement of the conduction tissues, that is, of the phloëm and xylem, is the reverse to that found in the normal root. The nodules in some species of legumes grow rapidly to full size and then stop growing. There is, in such nodules, no permanent meristematic tissue. In others, a group of growing cells remain active near the tip of the nodule. This meristematic mass con-

tinues growing and may branch from time to time. Soon plants, in consequence, show forked nodules sometimes resembling masses of coral. Certain well-differentiated areas apparently are always set apart for the growth of the microörganisms. The nuclei of the invaded cells eventually undergo degeneration. Stained preparations made from nodules show that in the older cells at least, most of the bacteria have assumed involution shapes resembling in many respects those found in culture media. These were termed *bacteroids* by the earlier investigators before their true bacterial relationship was understood. The morphology of the organism seems to vary somewhat with the age of the nodule and the species of legume.

Comparative studies of legumes grown in soils which have been sterilized and freed from *Rhizobium leguminosarum,* consequently having no nodules upon the roots, with plants having nodules prove conclusively that the organism fixes atmospheric nitrogen and in some manner passes this on in combined form to the plant, that is, to the legume. Legumes are, therefore, not dependent merely upon soil nitrogen, but by the aid of the bacteria can utilize the atmospheric nitrogen for their development. There seems to be no reasonable doubt but that the energy required for fixation of the nitrogen by the bacteria is furnished by the plant through carbohydrates, probably sugars, which are supplied. In just what manner the legume secures the nitrogen through the help of the bacteria, is somewhat less certain. It has been assumed by some writers that the bacteria fix the nitrogen and use it in building up their own protoplasm, and that only after the death of the bacterial cell and its digestion, either as a result of autolysis or as a result of the secretion of enzymes in the nodules by the plant, the digested products may be absorbed by the legume. Other investigators have concluded that a part, at least, of the nitrogen fixed is excreted by the bacteria in the

form of some soluble compound and that the plant can benefit by the presence of the bacteria without destroying them.

It already has been noted that there probably should be recognized more than one species of the genus *Rhizobium*. This is evidenced particularly when attempts are made to inoculate legumes of one kind growing in sterile sand with the organisms originating from the root of some other species. In general leguminous species of plants which are closely related botanically may be cross-inoculated readily. For example, the various species of the genus *Medicago*, to which the alfalfa belongs, readily cross-inoculate. Furthermore, bacteria from the roots of sweet clover belonging to the closely related genus *Melilotus* also cross-inoculate readily with alfalfa. Forms, however, so distantly related as soy beans, lupine, and the clovers seem quite impossible of cross-inoculation. It is entirely probable that the organisms producing the nodules on these three plants belong to different species.

It is evident from the preceding discussion that although bacteria capable of causing root nodules to form on the roots of leguminous plants are widely distributed and present in most soils, it is nevertheless true that in many soils those particular forms, possibly species, capable of inoculating the roots of certain legumes may be absent. Care must be used, however, to differentiate between the lack of organisms capable of inoculating roots and physical or chemical soil conditions unfavorable to the growth of plants. Many of the clovers, for example, prefer to grow in a soil containing a considerable quantity of lime, one which is, therefore, neutral or somewhat alkaline in reaction. With such plants, lack of a good stand may be quite as apt to be due to wrong soil conditions as to the lack of the proper inoculating organism. Various methods of artificial introduction of organisms capable of inoculating the roots of certain

species of legumes have been suggested. Soil from a field in which a particular legume has been successfully grown is often used for the inoculation of other fields, sometimes being spread at the same time that the seeds of the legume are sown. This method has the obvious disadvantage of great bulk and the chance of introducing undesirable weeds. The second method commonly practiced is that of the use of pure cultures. Some of the agricultural experiment stations in the United States and Canada, as well as certain commercial firms, supply these regularly, usually in the form of a liquid culture. It is customary to supply a culture of the organism isolated from the nodules of the particular legume which it is desired to inoculate. In some cases it is necessary for the organism to be increased. A culture medium containing phosphate, sugar, and water may be inoculated with the culture of the organism it is desired to grow, and within a few hours the numbers of bacteria will greatly increase. Inoculation may be effected by moistening the seeds which are to be sown with such a culture or in some cases by mixing with dry soil and spreading this upon the ground to be inoculated.

Much has been written in bacteriological literature concerning the possibility of exalting or increasing the nodule-producing power of particular strains of *Rhizobium leguminosarum*. This increased power of nodule formation has been termed *virulence*. It is claimed by certain manufacturers and by some authors that it is possible by growing in certain kinds of culture media, and by subjecting the organisms to suitable environment, to increase or decrease this virulence. Adequate scientific demonstrations of such variations in virulence have apparently not been made. It seems that quite as wide variations in inoculating power may be found among various cultures isolated from different plants of the same legume species.

Inoculation of soil for growing bacteria has been widely

practiced. When once the bacteria are thoroughly inoculated through the soil, that is, after there have been one or more successful crops of legumes grown on a particular area, the organisms will persist for a considerable period of time even though the particular legume is not present.

Nitrogen Fixation by Symbiotic Fungi.—Many kinds of higher plants are found to have growing in or upon their roots species of fungi apparently intimately connected with the metabolism, life history and successful development of the plant. The plants which show this characteristic are in many cases inhabitants of swamps and bogs, others are the so-called epiphytes or air plants. In addition, however, many species are known to possess these fungi upon their roots, though they grow under normal soil conditions. Fungi growing in or upon plant roots are generally termed *mycorrhiza.* In some species the fungi occur inside the root, frequently giving rise to swellings, nodules, tuber formation, etc. In other cases the fungi grow over the surface of the root, penetration, if any, being relatively shallow. The former are termed *endotrophic* and the latter *ectotrophic.*

Among the endotrophic mycorrhizas are some which grow in the roots of orchids. All members of this great plant family (*Orchidaceæ*) show a certain zone in the roots just inside the epidermis given over to hyphæ of a fungus. It is found that seeds will die soon after germination, if this fungus is not present and functioning. The roots of the Russian olive tree, furthermore, and its close relatives, such as the shepherd berry, have nodules on their roots much resembling the nodules of legumes, but harboring an endotrophic fungus. The same is true of the roots of the alder and the New Jersey tea of our prairies. Apparently these nodules are normal to the plant and the organisms growing in them are in some respects beneficial. In certain species of flowering plants which have lost the power to produce chlorophyl, such as the Dutchman's pipe, apparently the

task of taking up plant food from the soil and elaborating it for the use of the plant is largely given over to organisms growing in the tuberous or nodular roots. Sufficient work has not been done with most of these plants to prove conclusively that the primary function of the mold is to fix atmospheric nitrogen. There is some evidence pointing to this as a fact for certain forms such as the alder, while experiments upon New Jersey tea did not lead to the same conclusion. Apparently there is a condition of symbiosis. The exact benefit reaped by the plant has not been determined with certainty.

The roots of many trees, and particularly plants growing in bogs and swampy places, are covered with the mycelium of a fungus or so-called ectotrophic mycorrhiza. In certain groups of plants, such as those belonging to the genus *Vaccinium,* which includes the huckleberries, blueberries and cranberries, practically no growth will occur, at least normal development will not take place unless the fungi are present upon the roots. In other cases it is apparently true that the mycorrhiza is largely parasitic and more harm is done than good. Certain of the toadstools and mushrooms which appear in woods are of this character and the hyphæ may be traced from the base of the fruiting body to the tip of the tree roots.

In summary, it may be concluded that many plants not belonging to the group of legumes depend, in part at least, upon the activity of molds growing in their roots or in root nodules, for their best development. It is not at all improbable that in some cases nitrogen is fixed and furnished to the growing plant by these organisms, but adequate proof has not been brought forward in most cases.

Nitrogen Fixation by Nonsymbiotic Bacteria.—It has been abundantly demonstrated that there are several, probably many, kinds of bacteria living in the soil not in symbiosis with higher plants, which are capable, under certain condi-

tions at least, of fixing atmospheric nitrogen and utilizing it for their own metabolism. Some species of these are anaerobic, most are aërobic. As noted above with *Rhizobium*, it is customary to rate the rapidity and amount of nitrogen fixation by comparing the number of milligrams of nitrogen fixed with the number of grams of sugar utilized as energy, for these organisms are just as dependent as are the symbiotic type upon some easily oxidizable substance, such as carbohydrates, from which they may secure energy. The group of organisms which has been most studied and is probably, on the whole, most important in such nitrogen fixation, is the one whose members belong to the genus *Azotobacter*.

Azotobacter.—The genus *Azotobacter* includes those soil organisms which are aërobic, secure their growth energy almost entirely by the oxidation of carbohydrate material and which are capable of fixing atmospheric nitrogen. The cells usually are somewhat larger than those of other soil bacteria. Under favorable conditions in the laboratory in suitable media, they may appear almost yeastlike. They are usually spherical, ellipsoid, or in some cases elongate. Some species are motile, usually by means of polar flagella. Others are nonmotile. Some species possess the power of producing a diffusible pigment, greenish or brown in color. Species belonging to this genus are widely distributed in nature and about ten have been described in the literature.

The species first described, and one of those most commonly met with is *Azotobacter chroöcoccum*. This and another species known as *Azotobacter Beyerincki* are probably the most widely distributed in soils. They grow readily upon laboratory media containing phosphate and certain other inorganic salts plus some easily available source of carbon, particularly sugars and higher alcohols. They do not develop in media or in soils having an acid reaction. One species of *Azotobacter, A. Vinelandii*, de-

scribed by Lipman in New Jersey, has been found able, in the laboratory, to fix from fifteen to twenty milligrams of nitrogen per gram of sugar or alcohol (such as mannitol) used. The amount of nitrogen fixed is also influenced by other constituents of the medium. It has been shown by certain authors that the addition of soil extract, particularly of humates, exerts a decided stimulating action. It has been found that under certain conditions species of *Azotobacter* growing together with algæ are capable of fixing considerable quantities of nitrogen, apparently there existing symbiotic relationship between the two types of organisms.

Efforts have been made to utilize cultures of *Azotobacter*. Most fertile soils contain bacteria of this type in abundance. The addition of such organisms, therefore, is superfluous in such soils and useless in soils that are not adapted to their growth.

Organisms belonging to this group are not of such immediate value to the farmer as are those growing in symbiosis with leguminous plants. Nevertheless, in the long run they are probably responsible for the accumulation and storing up of large quantities of nitrogen in the soil. It should be noted, however, that there is no evidence that these bacteria take up free nitrogen and turn it over to plants growing in the soil. It is probable that they utilize the nitrogen in their own metabolism and it is only after the death and decay of the *Azotobacter* cells that the nitrogen becomes available to higher plants. Inasmuch as these bacteria can utilize sugars, and salts of various organic acids as sources of energy in the laboratory, it is probable that they secure their energy from the same sources in the soil. Their development, therefore, is dependent upon an abundant supply of such materials.

Anaërobic Nitrogen-fixing Bacteria.—Several species of anaërobic bacteria have been described as capable of fixing atmospheric nitrogen. The most important of these is

Clostridium pasteurianum. While this type of fixation may be shown in the laboratory it is not probable that it is of any great economic significance. The amounts of nitrogen fixed are not large and the utilization of carbohydrate as a source of energy must be much greater under anaërobic than under aërobic conditions. Such organisms are, therefore, far less efficient nitrogen-fixing machines than are the aërobic species such as *Azotobacter.*

Nitrogen Fixation by Nonsymbiotic Molds.—Considerable study has been devoted to the question of whether or not molds not living in symbiosis with plant roots are capable in the soil of fixing atmospheric nitrogen. There is some diversity of opinion, but on the whole it may be said that it has not been definitely proved that molds do possess this capacity. However, under certain soil conditions, such as in the leaf mold occurring in forests, molds are undoubtedly very important in breaking down organic compounds and it is not impossible that some species may be found capable of fixing atmospheric nitrogen, adding thereby to the fertility of forest soils. Such has not been definitely proved.

The Cycle of Carbon

The common forms in which carbon is found in nature are as the element, (that is as carbon), as carbon dioxide, and as relatively complex carbon compounds, the so-called organic compounds. The cycle of carbon, therefore, is not as complex as that of nitrogen, and many species of organisms are known which can bring about transformations of carbon from one compound to the next.

Carbon dioxide, as it exists in the atmosphere and in solution in soil water, is taken up by green plants and these, by utilizing the energy from the sun's rays, are capable of forcing this into combination with hydrogen, producing formaldehyde, and then polymerizing this compound into carbohydrates, first into sugars and then into starch. Some

bacteria are apparently also capable of taking up atmospheric carbon dioxide or carbon dioxide in solution and utilizing the energy secured by oxidation of inorganic compounds to assimilate the carbon dioxide into their own protoplasm. Such organisms have already been noted among the so-called nitrifiers, the energy secured by oxidation of ammonia, for example, being in part used for the reduction of carbon dioxide and the production of organic carbon compounds.

Microörganisms in general (as well as animals) possess the ability to break down complex compounds of carbon, securing growth energy by their oxidation, and giving off carbon dioxide continuously. In the main, therefore, the carbon cycle consists in manufacture of carbon compounds by green plants and their oxidation not only by these plants but also by animals and microörganisms.

Under certain anaërobic conditions, microörganisms may attack carbon compounds, evolving hydrogen and carbon dioxide. In general the carbohydrates have the empirical formula $C_nH_{2n}O_n$. It is apparent, therefore, that if free hydrogen gas is given off together with carbon dioxide that there will be a residuum of carbon. Apparently changes of this kind have been responsible for the formation of peat and of coal.

Production of Carbon Dioxide in Soil by Microörganisms.—Practically all of the aërobic bacteria and many of the anaërobic bacteria produce carbon dioxide in the decomposition of carbonaceous material. It is, therefore, constantly being produced in the soil, and is of considerable significance there. A large proportion of the carbon dioxide evolved escapes in the air, some portions are dissolved in the soil water and in part may be taken up by plant roots. It is evident that the soil solution must, under most conditions, be practically a saturated solution of carbon dioxide. This exerts a marked solvent action upon many of the minerals

present in the soil and undoubtedly is one of the important factors in bringing about the solution of materials necessary for the growth of higher plants.

The Decomposition of Insoluble Carbohydrates in the Carbonaceous Matter in the Soil.—A considerable proportion of the carbonaceous material added to the soil is in the form of cellulose and closely related compounds. This is not readily attacked by most kinds of bacteria, but there exist in the soil certain specialized forms which decompose cellulose readily. Members of the genus *Actinomyces* are particularly active in this respect, causing rapid digestion of cellulose. Certain molds are also active, particularly in acid soils. Then, too, a considerable number of species of bacteria have also been isolated from the soil, capable of bringing about cellulose digestion. They belong to a variety of genera. They may be recognized in the laboratory by their ability to grow upon an agar containing finely divided cellulose and to produce a clearing of the medium. By the destruction of cellulose in the soil, decomposition products such as the simple soluble carbohydrates and organic acids, are produced which may be utilized by many other soil forms.

The changing organic matter present in soil is usually termed collectively "the soil humus." Most plant residues added to soils are comparatively high in carbon and poorer in nitrogen. In the process of decomposition carbon, in the form of carbon dioxide, is given off more rapidly than is nitrogen. In consequence, there is a tendency for the humus, as it becomes older, to show a smaller ratio of nitrogen to carbon. This carbonaceous material present in soil is of significance from many points of view. It ameliorates the physical condition of the soil and furnishes directly and indirectly from the products of its decomposition food material for many of the nitrogen-fixing and other soil bacteria. It is made up in part, of course, of the bodies of

bacteria and fungi themselves. Its color probably is in part determined by the pigments produced by the bacteria and the molds.

The Cycle of Phosphorus

Phosphates are quite essential to the development of most higher plants. They are usually present in soil in the form of tricalcium phosphate which is quite insoluble. It must be converted into soluble phosphate to be taken up by plant roots and combined into organic compounds such as the phosphoproteins, nucleic acid, etc. The solution of the insoluble phosphate in soil is probably in large part the result of the action of organic acid and the carbon dioxide produced as the result of the growth of microörganisms and of the formation of nitric acid from ammonia. There seems to be good evidence that rapid nitrification in the soil brings about a considerable solution of the insoluble phosphates, rendering them available for plant growth.

Phosphates also have a relationship to the activity of soil microörganisms in their direct stimulating effect upon growth. Many species of bacteria bring about fermentative changes much more rapidly in the presence of phosphates than in their absence. This is most certainly true with the bacteria which are capable of fixing atmospheric nitrogen.

The Cycle of Sulphur

Sulphur in nature is found in its free form, as sulphates, sulphides and in organic compounds, particularly in certain classes of proteins and in certain essential oils, such as mustard oil. Microörganisms are active in bringing about the various transformations of sulphur from one condition to another. Protein compounds are broken down into amino acids, particularly the amino acid cystein, and from this is formed hydrogen sulphide. This may be oxidized to sulphates. Sulphates are taken up by the plant

roots and built up again into protein. Under anaërobic conditions sulphates may be changed into sulphides. Certain bacteria are also known which can produce free sulphur, and others which can oxidize free sulphur to sulphates.

Organisms Oxidizing Hydrogen Sulphide and Free Sulphur.—A large group containing many families and numerous genera of the so-called purple or sulphur bacteria (thiobacteria) are known which evidently utilize hydrogen sulphide, very largely in their metabolism. Many springs and ground waters contain considerable amounts of sulphides which, upon aëration, give off free hydrogen sulphide. Bacteria belonging to this group are active in bringing about oxidation in such waters. Among the most common forms are members of the genera *Beggiatoa* and *Thiothrix*. These organisms, by the oxidation of hydrogen sulphide, produce granules of free sulphur inside of the cells. When examined microscopically these free sulphur granules will be noted as glistening globules. If such bacteria are dried upon a slide and a small amount of a sulphur solvent, such as carbon disulphide is added and allowed to evaporate, sulphur crystals may be detected. These bacteria also possess the power of oxidizing the free sulphur to sulphates. It is not probable that these organisms are particularly significant in the soil as hydrogen sulphide is rather readily oxidized to sulphates by chemical means and by various catalytic agents in the soil. The species of bacteria which may bear some relationship to this phenomenon in the soil are not well known. The process has been sometimes termed *sulphofication*.

Bacteria belonging to this group may be of some significance in the destruction of concrete structures such as cement tile through the formation of sulphuric acid and consequent disintegration.

Sulphates are quite as essential to plant growth as are

phosphates. Most soils are somewhat higher in sulphates than in phosphates and consequently additions of sulphates are not as frequently required for soils as are the phosphates. In some cases, however, they are deficient.

Organisms Reducing Sulphates to Sulphides.—Under anaërobic conditions, in the presence of organic matter, many species of microörganisms are known which will change sulphates to sulphides, in many cases releasing free hydrogen sulphide. This power particularly is possessed by many of the soil bacteria and by forms belonging to the genus *Vibrio*, particularly the species *Vibrio desulphuricans*.

Bacteria of this type may be of significance in soils which are water-logged and which contain an abundance of sulphates and organic matter. Hydrogen sulphide is quite poisonous to many plant roots. The reduction of the sulphates to sulphides, therefore, will make conditions particularly unfavorable for the development of many kinds of plants. Processes such as the reduction of sulphates to sulphides and of nitrates to nitrites are among the agencies which are important in causing plants such as corn to turn yellow when the soil in which they are growing becomes water-logged. These organisms are also important in sewage disposal. When organic matter, such as sewage, is mixed with a city water supply high in sulphates, such as magnesium sulphate, the reduction to sulphides and the formation of hydrogen sulphide may be so great as to create a nuisance. Special methods of sewage disposal have been adopted in some cases in which the sewage is more thoroughly aërated than is necessary with sewage which is lower in sulphates.

The Cycle of Iron

Iron in nature occurs in organic form and in the inorganic form in the oxidized and reduced condition. Micro-

organisms are known which oxidize the reduced iron to the ferric condition, and other organisms which, under anaërobic conditions in the presence of organic matter will reduce ferric iron to ferrous iron. The latter are of some practical significance as the ferrous iron compounds may be somewhat poisonous to plant roots.

Bacteria oxidizing ferrous to ferric iron are of considerable importance in the deposition of iron ore. It is probable that most of the great iron ore beds, consisting of iron oxide and iron carbonate, have been formed through the action of microörganisms. Bacteria belonging to this group for the most part are forms which oxidize the soluble salts of ferrous iron to an insoluble ferric condition and deposit them in the sheaths of the filaments. In the city water supplies they may accumulate in quantities such as to form reddish or yellowish deposits in the pipes, the so-called deposit of iron rust.

SECTION V

MICROÖRGANISMS AND DISEASE

CHAPTER XXI

DISEASE, INFECTION, AND RESISTANCE

Disease.—Disease may be defined as any abnormality in form or functioning of the body or of any of its parts. Thus defined the term includes many conditions frequently not thought of as disease. It includes, for example, conditions usually termed *traumata*, that is, the changes resulting from mechanical injury, such as a blow, a bruise or a cut. In addition to the traumatic diseases there are so-called *dietary* or *deficiency* diseases. These are known both in plants and in animals. A lack of sufficient iron, for example, will, in certain plants, result in a whitening of the leaves, that is, in a decrease in the amount of chlorophyl present. In man and animals diseases sometimes result from an improper balance among the principal food constituents: fats, proteins and carbohydrates. Still more frequently occur those diseases due to the lack of certain so-called accessory substances or vitamines. Such, for example, are the diseases scurvy, beriberi and probably pellagra. Still another group of diseases is due to the improper functioning of certain glands of the body such as goitre and diabetes. There may be also hereditary defects such as color blindness or congenital defects such as hare lip or clubfoot. All the conditions enumerated may be grouped together as *noninfectious* diseases.

An *infectious disease* is one caused by some microörganism. It is evident, therefore, that the primary grouping of diseases may be made into infectious and noninfectious.

The student in bacteriology is interested in the former and in the bacteria, yeasts, molds and protozoa which may produce them.

Among the infectious diseases of man the following are important: pneumonia, tuberculosis, boils, abscesses and bone infections, cerebrospinal meningitis, gonorrhea, Malta fever, anthrax, gaseous gangrene, tetanus, bubonic plague, glanders, paratyphoid, typhoid, dysentery, diphtheria, influenza, smallpox, chicken pox, measles, hydrophobia, mumps, Asiatic cholera, syphilis, malaria, yellow fever, trench fever, typhus fever.

Among the infectious diseases of domestic animals and birds may be noted the following: strangles or distemper, glanders, infectious abortion, fistula, poll-evil, wound infection, tetanus, hemorrhagic septicemia, pseudotuberculosis, dourine, horse sickness, swamp fever, pox and pneumonia in the horse; wound infection, mastitis, pneumonia, milk fever, blackleg, malignant edema, hemorrhagic septicemia, infectious abortion, pseudotuberculosis, lumpy jaw, surra, Texas fever, pleuropneumonia, foot and mouth disease, rinderpest, cowpox and anthrax in cattle; hog cholera, swine plague, swine erysipelas, hemorrhagic septicemia, anthrax, tuberculosis, foot and mouth disease in the hog; anthrax, pneumonia, Malta fever, pseudotuberculosis, sheep pox, in goats and sheep; fowl cholera, fowl plague, fowl typhoid, white diarrhea of chicks, roup, tuberculosis, spirochætosis, aspergillosis, epithelioma contagiosum in domestic fowls; and rabies and dog distemper in the dog.

Infectious diseases among plants may be differentiated into those caused by fungi and those caused by bacteria. A large number of these latter have been described under the name of *bacterioses*. Among the more common may be noted the fire blight of the apple and pear, the black rot of cabbage, crown gall of apple and other fruits and various blights, galls, tumors, leaf spots and wilts of various plants.

Certain infectious diseases of insects are also of economic importance. The best known of these are the flacherie of the silk worm, American foul brood and European foul brood of bees.

Contagious and Noncontagious Diseases.—A *contagious* disease is one which is more or less readily transmissible from one individual to another by direct or indirect contact. A *noncontagious* disease is one that is not thus transmitted. In practice the term contagious is usually modified. We speak of certain diseases as being highly contagious, of others as being moderately or slightly contagious. At the other end of the series we have diseases which are noncontagious. Particular notice may be drawn to the fact that the terms *infectious* and *contagious* are frequently confused in popular literature and discussion. It will be noted that infectious refers to cause, contagious to method of spread.

Factors Predisposing to Disease.—The resistance of an individual to a disease undoubtedly varies from time to time. It is probable that unusual fatigue, hunger, unusual chilling or excessive heating of the body may increase the susceptibility of the individual. Certain diseases are more apt to attack children, others more frequently attack adults. There are also diseases characterized usually as diseases of old age.

Heredity and Disease.—To be inherited, in the strict sense of the term, a disease must be of a character such that it can be transmitted indefinitely from one generation to the next. Certain defects, such as color blindness, may be transmitted in this fashion, but this is not true of any of the infectious diseases. Animals may at birth occasionally be infected with certain diseases acquired by them from the mother before birth. Such diseases are, however, not inherited in the strict sense of the term.

Immunity

Immunity may be defined as resistance to disease. Its converse is *susceptibility*. The general fact that individuals vary greatly in their ability to resist disease has of course been known since history began. Many explanations were suggested to explain the reason for an individual becoming immune to a disease after having once been attacked by it, as by smallpox, for example. Some five of these theories are worthy of note. Three of the earlier theories have been quite generally discarded as imperfect or not tenable. Two of the others, however, have played a very large part in the development of modern views.

Exhaustion Theory of Immunity.—When it was recognized that bacteria and other microörganisms sometimes cause disease it was suggested that an individual became immune to the disease caused by such an organism due to the using up or abstraction from his body of some of the material essential for the growth of the organism. As long as this deficiency existed bacteria of that type could not grow in the body. It was necessary to discard this conception, however, when it was shown that the blood, for example, of individuals entirely immune to certain diseases contained all of the nutrients necessary for the active growth of their causal organisms.

Noxious Retention Theory.—When bacteria are cultivated in the laboratory, it is always noted that they grow most rapidly in the early stages of development. As time progresses the growth becomes slower and slower and finally ceases. It was suggested early that this stopping of growth in culture media was due to the development of substances injurious to the organism. Bacteria growing in milk, for example, produce lactic acid in sufficient quantities eventually to stop the growth of the organism producing the acid. Reasoning by analogy it was then suggested that bacteria

growing in the body and producing certain diseases might develop there substances not injurious to the health of the individual, but which would prevent further growth of the microörganism in question. Studies of the blood, however, soon revealed this suggestion to be fallacious. Immunity apparently is not due to the retention of noxious substances produced by the microörganisms causing the disease.

Pythogenic Theory of Disease.—It was also suggested that filth and decaying organic material might be the cause of disease. A half century ago it was believed demonstrably true that malaria was due to the inhalation of the miasms arising from the decomposition of organic matter in swamps. Murchison, in England, contended that filth and accumulated dirt, particularly decaying organic matter, produced many types of fever. When the germ theory of disease was suggested, the pythogenic theory was modified and it was contended that pathogenic or disease-producing microörganisms might be generated in such decaying heaps of organic substances, gain access to the air, and produce disease as a result of inhalation. It was even contended that disease germs might originate spontaneously in such material. While we now know that disease may sometimes be spread as a result of the accumulation or presence of filth and dirt, nevertheless, it has been quite definitely proved that these factors do not stand in the causal relationship to disease which was suggested by the proponents of the pythogenic theory.

Ehrlich's Humoral Theory of Immunity.—As a result of detailed studies Ehrlich came to the conclusion that immunity is due to the development by the body tissues of substances which will in some way antagonize the invading microörganisms or neutralize their poisonous and deleterious products. The substances thus produced in the tissues he termed *antibodies*. An individual, therefore, becomes immune or is naturally immune to disease because of the

sufficient presence of the appropriate type of antibody in the blood or in the body tissues. This theory has apparently stood the test and there is little reason to doubt that immunity in many diseases, probably in most, is due to the presence or to the development of antibodies which can specifically antagonize the invading microörganism.

Metschnikoff's Theory of Phagocytosis.—As a result of the microscopic examination of the blood of animals which had been injected with various kinds of bacteria, Metschnikoff came to the conclusion that certain body cells, particularly certain types of white blood corpuscles, were capable of engulfing and destroying invading microörganisms. He concluded that immunity was due to the power of these cells to destroy bacteria. An individual who became immune to disease had, so to speak, trained his phagocytic body cells to destroy the particular type of organism in question. Such an individual, therefore, would be immune to the disease as long as this function on the part of the white blood cells persisted. It will be shown later that Metschnikoff's theory contained much of value. Undoubtedly the white blood cells are important in preventing disease, and any adequate explanation of immunity must take into consideration their activity. However, it has been found that the white blood cells by themselves usually are relatively incapable of destroying a microörganism but must act in conjunction with certain antibodies present in the blood stream. It will be noted, therefore, that Ehrlich's theories and Metschnikoff's theories have been finally united and together constitute the basis for the modern science of immunology.

Kinds of Immunity.—Common observation teaches that man is immune to many diseases which attack animals. For example, man does not contract hog cholera or dog distemper. Most diseases of plants are not transmissible to animals or *vice versa*. Many diseases of man cannot be

transmitted to lower animals. Within a certain species, furthermore, certain individuals are found from birth to be much more resistant than other individuals to a particular disease. All of these are examples of *natural*, that is, congenital, immunity.

An immunity, on the other hand, which develops after birth is said to be *acquired*. It may develop as the result of any one of a considerable variety of conditions. The body itself may take an active part in the development of immune substances, that is, of antibodies. Such an immunity is termed an *active immunity*. Such, for example, is the immunity which develops as a result of having had smallpox or diphtheria. Immunity of this type, furthermore, results from vaccination, that is, inoculation with dead or attenuated cultures of microörganisms causing the disease, against which it is desired to immunize. The blood serum of an actively immunized animal comes to contain antibodies specific against the disease.

The blood serum of an actively immunized animal may be injected into an animal which has not been immunized and such an individual may acquire, as a result, a considerable degree of immunity. In this case, the tissues of the individual into which the serum is inoculated or injected do not play any part in the manufacture of the antibodies, that is, the body is passive. This type of immunity, therefore, is termed *passive immunity*. The immunity conferred as a result of the injection of antidiphtheritic serum is of this type, likewise the immunity conferred by the injection of anti-hog cholera serum.

An acquired immunity may be either permanent or temporary. In general an active, acquired immunity is somewhat more permanent than a passive acquired immunity. For example, hogs which are passively immunized against hog cholera by the so-called single treatment, that is, by the injection of an anti-hog cholera serum, acquire an im-

munity which is distinctly temporary. The animal is protected for only a few weeks. By the use of the vaccine, however, the animal may be caused to develop an active immunity which will last for a much longer perior of time, probably for years in many cases.

Factors Which Determine Immunity.—The factors which determine the resistance against disease in plants and animals may be grouped under two general headings: those which are of importance in what may be termed *general resistance,* and those of importance in *specific resistance* against particular diseases.

In animals general resistance is due in large part to the coverings of the body and to the membranes lining its respiratory and digestive tract. The *skin* is an effectual barrier to the entrance of microörganisms generally. The *mucous membrane* of the nose and respiratory tract constantly throw off mucus upon which dust and microörganisms inhaled generally catch, and are pushed up toward the body openings into the throat and nose by the cilia of the epithelial cells. In other words, a cleansing stream of mucus is constantly rising from the lungs and prevents the accumulation of dust particles in this organ. In the alimentary tract the *digestive juices,* particularly the strongly acid gastric juice, destroy many microörganisms. The subcutaneous tissues, particularly the layers of *fascia,* lying parallel to and just under the skin in many parts of the body and separating muscles and subdivisions of tissues from each other, are important agencies in preventing the distribution of microörganisms through the body.

In plants the general resistance is usually due to the presence of bark or cuticularized layers covering the living tissues. Many plants also contain substances, such as acids, tannins, glucosides, etc., which prevent the development within them of most kinds of microörganisms.

Specific resistance in animals, as already noted, depends

upon the development, in the tissues of the animal, of antagonizing substances termed antibodies. Any substance which, when injected into the tissues or when present in the tissues, causes these tissues to develop an antibody is termed *antigen. A consideration of specific immunity, therefore, is primarily a discussion of the various substances or antigens which can incite the tissues of the body to the development of immunizing substances and a study likewise of these immunizing substances or antibodies.*

The following table lists the more important of the antigens and their corresponding antibodies, which will be discussed on the succeeding pages:

ANTIGENS AND THEIR CORRESPONDING ANTIBODIES

ANTIGENS	ANTIBODIES
Toxin	Antitoxin
Bacterial cell (agglutinogen)	Agglutinin
Soluble protein (precipitogen)	Precipitin
Cells foreign to body:	Cytolysin
Bacteria	Bacteriolysin
Red blood cell	Hemolysin
Bacterial cell	Opsonin

Certain kinds of bacteria capable of producing disease are known to produce poisons termed *toxins.* The body develops immunity against such by the production of *antitoxins.* When certain kinds of bacteria or other cells gain access to the body, the latter may sometimes produce substances which will cause these bacteria to clump together or *agglutinate.* These are termed *agglutinins.* Foreign proteins, that is proteins not native to the body of the animal into which they are injected or coming from another species of animal or plant, will cause an individual injected to develop substances in the blood serum which will *precipitate* the corresponding protein. Such antibodies are termed *precipitins.* The presence of foreign cells (such as bacteria) may cause the tissues to develop substances which will digest or dis-

solve the particular organism in question. A person who has completely recovered from typhoid fever, for example, will generally contain in his blood serum substances which will dissolve or digest typhoid bacilli. Such substances or antibodies are termed *bacteriolysins.* Sometimes another type of antibody may be developed against bacterial or other cells. These substances function by making the bacteria more attractive apparently to the white blood cells. In other words, they enable the white blood cells to destroy the microörganism. They are termed *opsonins.* Our consideration of the factors underlying immunity, therefore, must include a brief consideration of these various antibodies and their corresponding antigens.

Toxins and Antitoxins

A toxin may be defined as an organic poison, that is, a poison produced by living cells of plants or animals and having certain characteristics of which the following are most important:

1. Toxins are very easily destroyed (labile). Particularly are they thermolabile, that is, easily destroyed by heat. They deteriorate rapidly when kept in the light and in the presence of moisture.

2. When toxins are injected into suitable animals in amounts smaller than sufficient to cause death (sublethal doses) these animals will respond by the development in the blood serum of antitoxins or substances which will neutralize the toxins. No poison, therefore, is a true toxin which will not cause animals to produce antitoxin to neutralize it.

It will be noted that the above definition does not include all organic poisons. The immunity resulting from the development of antitoxins in the body must be carefully differentiated from so-called drug habituation. Repeated injection of morphine (an organic poison), for example,

will cause the body to become accustomed to its presence and to endure relatively large amounts of the material. This resistance, however, is not due to the presence or formation of substances which definitely neutralize the activity of the morphine.

The exact chemical constitution of none of the toxins has thus far been determined. Generally when injected into an animal they require a period of incubation before the results of the poisoning action are manifest. In general they injure the body probably by combining chemically with certain of the body cells or tissues. The toxin produced, for example, by the bacillus causing tetanus or lockjaw combines with the central nerve cells, injuring them and producing thereby the symptoms of the disease.

Sources of Toxins.—A relatively small number of the disease-producing bacteria develop toxin. The most important of these are the bacteria which produce the diseases diphtheria, tetanus and botulism (one type of food poisoning). The organisms causing blackleg in cattle and gaseous gangrene in man and certain types of dysentery bacilli develop toxins likewise. It is evident, inasmuch as toxins are produced by only few of the pathogenic bacteria, that antitoxins for immunizing against or curing such diseases must be limited in number. There is no probability, for example, that an antitoxin can ever be secured for a disease such as tuberculosis in which there is no evidence of any toxin production by the bacteria.

These toxins are excretions of the bacterial cells. Certain other plant cells are also known which can produce toxins. The juice of the castor oil bean seed, the inner bark of the black locust and the seed of the jequirity bean, all contain toxins. Certain poisonous animals are also toxin producers. Snake venom, for example, contains one or more toxins. The stings of many insects, fish and other animals are injurious because of the presence of true toxins.

Origin of Antitoxin.—It has already been noted that when a toxin is injected in sublethal doses into a suitable animal, this animal will react eventually by the development in its blood serum of substances (called antitoxins) capable of neutralizing toxin of the type injected. Experience has shown that these antitoxins are developed not only in quantities sufficient to neutralize the amount of toxin injected, but quantities greatly in excess of this. It was also noted that toxins poison because of their ability to unite chemically with certain cells of the body and injure them. According to the Ehrlichian conception, when these toxins are not combined with the cells in amount sufficient to cause death of the cells, these cells are stimulated to an excessive production of antitoxins which are thrown off into the blood serum and by combining there with the toxin prevent its union with the cells. It may be emphasized that antitoxins are specific, that is, each kind of toxin causes the development in the animal's body only of its own particular type of antitoxin and this antitoxin will neutralize only the type of toxin which caused its production.

Manufacture of Diphtheria Antitoxin.—The blood serum from an animal which has been actively immunized by repeated injections of the toxin of the diphtheria bacillus may be used for the prevention or treatment of diphtheria in man. An account of the method of preparation will serve to show the general principles governing manufacture of antitoxins. Production of antitoxins of other types differs from that described for diphtheria only in details.

Preparation and Standardization of Toxins.—Inasmuch as the diphtheria toxin is an excretion of the diptheria bacillus it can best be prepared by growing the bacteria in broth and when the toxin formation has reached the maximum the bacteria may be filtered off by means of a porcelain filter. The broth is usually placed in large flat-bottomed flasks, the layer of broth being comparatively thin. The

diphtheria bacteria grow as a film over the surface and within a few days produce the maximum amount of toxin. Some substance, such as cresol, which will kill the diphtheria bacilli without injuring the toxin, is added and the broth filtered through a suitable porcelain filter. This broth containing the toxin is now ready for standardization. Inasmuch as no chemical test has been devised for the detection or estimation of a toxin it is necessary to standardize by injection of the material into a suitable animal. The animal of choice is the guinea pig. The unit of toxicity is determined by injecting varying amounts of the toxic broth into a series of guinea pigs weighing about 250 grams each. Those animals which die in just three days have received a minimum lethal (fatal) dose of the toxin. This unit of toxicity is usually abbreviated as the M. L. D. dose. A broth having a satisfactory content of toxin frequently will show one thousand or more M. L. D. per cubic centimeter, that is, each c.c. will contain enough poison to kill one thousand guinea pigs weighing 250 grams each.

Injection of the Toxin.—Several species of animals may be used in the production of diphtheria antitoxin. The horse has proved to be the most suitable from the commercial standpoint because it will yield a large quantity of serum at one bleeding and usually will develop an antitoxin of relatively high potency. The horses used must first be examined to make sure that they are free from disease and in good physical condition. They are then injected subcutaneously with a small amount of diphtheria toxin. This may cause some local swelling and fever. This soon disappears, however, and the animal may then receive a second dose of the toxin. At intervals larger and larger doses of toxin are injected until it is evident that the animal has reached a high degree of immunity and the blood contains considerable quantities of antitoxin. A small sample of blood is then withdrawn from the horse and tested to deter-

mine its antitoxin content. The horse is bled from the right jugular vein by means of a sterile trocar and tube into a sterile glass vessel. Usually about one liter of blood may be secured per one hundred pounds weight of horse. This amount can be withdrawn from the animal without serious injury. After a period for recuperation the animal may then be injected several times more with diphtheria toxin and again bled. Successive bleedings from the same animal may thus be secured over a period sometimes of several years. The blood is allowed to clot, that is, the fibrinogen of the plasma is transformed into fibrin which catches the blood cells as in a net and by its contraction squeezes out the blood serum from the clot. This clear, straw-colored blood serum is found upon examination to contain the antitoxin.

Standardization of Antitoxin Serum.—Inasmuch as there is great variation in the relative antitoxic content of the serum secured from different horses it is necessary to standardize each sample. For this purpose, the antitoxin content is compared with a sample of standard antitoxin, secured in the United States from the Hygienic Laboratory of the Public Health Service in Washington, D. C. The standard serum secured from this source is diluted so that each cubic centimeter contains one immunity or antitoxic unit. It is evident that the antitoxin which has been manufactured cannot be compared directly with the standard antitoxin but only by use of titration of each of these against a toxin. The manufacturer, therefore, uses a suitable sample of toxic broth. This is first titrated against the standard antitoxin secured. To each one of a series of tubes one antitoxic unit of the standard serum is added. To each of these tubes, then, graduated amounts of the toxic broth are likewise added; to the first tube a small amount, to the next a larger amount and so on to the last. Each tube is then made up to the same volume with sterile physiological salt solution and the

contents thoroughly mixed. The content of each tube is then injected into a guinea pig weighing 250 grams. It is probable that the guinea pig receiving the injection of the contents of the first tube will survive, inasmuch as the amount of antitoxin is more than sufficient to neutralize all of the toxin. At the other extreme, however, it is probable that the guinea pig will die, inasmuch as the toxin present is more than completely sufficient to neutralize the antitoxin. It is evident, then, that the guinea pigs at one end of the series will live and those at the other end will die. Where these two series come together is an approximation of the neutralization point. The guinea pig, in other words, is used as an indicator in the titration of the toxin against the standard antitoxin. It is necessary arbitrarily to select the point of neutrality. That amount of toxin which when mixed with one immunity unit of the standard antitoxin and injected into a 250 gram guinea pig will kill that guinea pig in just four days is termed an L + dose. This amount of the toxic broth is now placed in each of a series of test tubes. That is, each test tube contains one L + dose of the now standardized toxin. To the series of tubes is then added graduated amounts of the antitoxin which has been manufactured and which it is desired to standardize. The contents of these tubes are in turn injected into suitable guinea pigs. Those animals receiving sufficient antitoxin completely to neutralize the toxin injected will live. Those receiving an excess of the toxin will die. Here again an animal may be chosen which dies in just four days. Inasmuch as it received an L + dose of toxin and yet died in just four days it must have also received one immunity unit of antitoxin.

The entire process may be compared to the standardization of an unknown alkali by use of a known alkali and unknown acid. The acid is first standardized against the known alkali, and then used for the standardization of the unknown alkali.

The standardized antitoxin is now filtered through porcelain filters and placed in suitable small containers. It may be used in prevention or curing of the disease diphtheria by injection subcutaneously by means of a hypodermic needle.

Preparation and Utilization of Other Antitoxins.—Antitoxins may be prepared in a somewhat similar manner for the prevention and treatment of tetanus or lockjaw, certain types of gaseous edema and for botulism. Antitoxins have also been prepared for the toxins of the venoms of various snakes.

Agglutinins and Precipitins

Two men working independently, Gruber and Widal, discovered almost simultaneously that when certain kinds of bacteria were present in the body, as in certain diseases such as typhoid, the blood serum came to contain some substance which would cause agglutination or flocculation of bacteria of this particular type. For example, when a drop of blood serum from a person who has typhoid fever is placed in a broth culture of typhoid bacilli and allowed to stand for a time, the bacteria gradually lose their power of motion, begin to clump together in masses which grow in size until finally they settle out, and the medium which was originally cloudy and turbid becomes clear with a flocculent sediment. Serum from normal individuals usually does not show this power. The substances developed in the blood capable of flocculating bacteria in this fashion were termed *agglutinins.*

Somewhat later the independent observation was made that whenever foreign proteins of any kind were injected into an animal, eventually the blood of that animal acquired the property of precipitating the corresponding or homologous protein. For example, if a dilute solution of egg white be injected into the blood stream of a rabbit at suitable

intervals, and after a time the rabbit bled, the blood allowed to coagulate and the serum secured, this serum, when added to a dilute solution of egg white will cause the medium to become opaque or cloudy and a flocculent precipitate will settle out. This will occur, however, only when the blood serum is mixed with the particular protein injected in the first instance. The substance produced in the blood serum is termed a *precipitin*.

Colloidal Solutions and Suspensions.—It was not at first recognized that the phenomena of agglutination and precipitation were fundamentally the same. Substances in extreme state of division or fineness are comparatively coarse colloids, or rather, their suspension constitutes a colloidal suspension. The protein molecule, such as that of egg white, apparently is large enough, or exists in aggregates which are large enough, to behave as suspended particles rather than as substances in true solution. Experience has shown that a great variety of substances added to particular proteins will cause precipitation, as in the so-called salting out process. A saturated solution, for example, of ammonium sulphate added to many proteins will cause them to go out of solution, or precipitate. The same is true with bacterial suspensions. A saturated solution of ammonium sulphate will also cause typhoid bacilli to flocculate out of suspension. This ability of certain salts to cause flocculation or agglutination is particularly well shown by mixing fine silt or clay with water. The solution will remain turbid almost indefinitely, that is, the particles will settle out very slowly indeed unless some salt, such as alum be added. When this is added, however, the clay particles bunch together, flocculate and settle to the bottom rapidly.

The resemblance between the phenomena of agglutination and precipitation by means of blood serum and the agglutination or flocculation by means of salts escaped notice for

many years. It has now been shown definitely, however, that the blood serum which is capable of causing flocculation of a particular kind of bacterium does this by sensitizing the bacteria to the action of certain salts. For example, common salt, that is, sodium chloride, in dilute solution does not cause flocculation or agglutination of typhoid bacteria. A dilute solution of serum from a typhoid patient with typhoid bacilli in distilled water will not cause agglutination. When, however, typhoid serum, bacteria and common salt are all present, flocculation or agglutination does occur. It can be readily shown that the agglutinin of the serum combines with the bacteria and makes these bacteria sensitive to the action of salt. Apparently the same explanation holds for precipitation as with proteins when a specific antiserum is used.

Agglutination.—Agglutinins are usually highly specific, that is, an agglutinin capable of causing clumping of one kind of microörganism will not cause clumping of other species. There are same exceptions to this rule, however. In some cases closely related bacteria will cause antibodies to develop which will cause some agglutination of the other species. In many instances, however, the reaction is so specific that it can be used readily in the diagnosis of disease. For example, the most common method of recognizing typhoid fever in the earlier stages of the disease is by the so-called *Widal test* (named after the man who first described typhoid agglutination). The test may be either microscopic or macroscopic. If the former, some of the diluted serum from the patient suspected of having typhoid is mixed with a drop of typhoid broth culture and examined under the microscope. If the serum contains agglutinin, that is, if the patient has typhoid, the bacteria will at first be observed to move about rapidly, finally their motion decreases, they begin to clump together and in a few minutes to half an hour comparatively few, if any motile bac-

teria are to be seen, the cells being arranged in large clumps. In the macroscopic test blood serum is added to a broth culture of typhoid bacilli or to a suspension of the typhoid bacilli in a physiological salt solution. When allowed to stand for a suitable length of time, the bacteria will flocculate out leaving a clear, supernatant liquid and a flocculent sediment.

Agglutinins apparently are not produced in quantities sufficient for assistance in recognition of many of the common diseases. The agglutination test is used most fre-

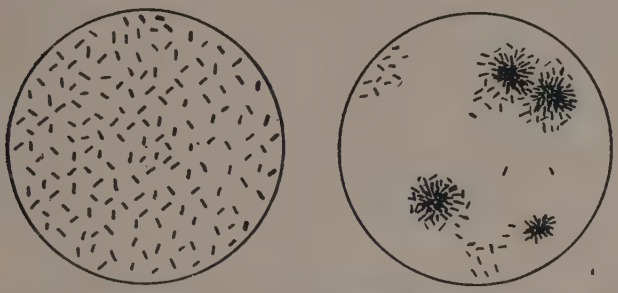

FIG. 60.—AGGLUTINATION. Left: Unagglutinated bacteria. Right: Clumped or agglutinated bacteria.

quently in typhoid, paratyphoid, dysentery, glanders, and for the differentiation of the various types of bacteria capable of causing pneumonia and meningitis.

Protein Precipitation.—Like the agglutinins the precipitins are highly specific. Precipitins developed for one kind of protein will usually not cause precipitation of other types. This property of precipitin formation upon the introduction of foreign protein has been used for the differentiation of proteins in several instances. By the injection of a particular protein into a suitable animal it is entirely possible to secure from that animal a serum which can thereafter be used as a specific test for the protein injected. This method is used in practice in the differen-

tiation of horse flesh and beef in countries where both are used. If some of the juice from known horse flesh is injected into a rabbit a serum can be secured which will always precipitate the juice from horse flesh. Similarly rabbit serum may be secured specific for other kinds of meat. To carry out the test some of the juice may be pressed from the sample of meat suspected of being horse flesh, treated with some of the serum from the immunized rabbit and the identification made by the absence or presence of a clouding or precipitate. In a somewhat similar manner the origin of blood stains has been determined. It is possible to secure an antiserum which is a specific precipitin for the blood serum of man or of any of the animals. The various flours (such as wheat and buckwheat) containing proteins may be separated from each other by means of such a test. The presence or absence of eggs in pastries may also be recognized. In some cases precipitin tests may be used in the recognition of disease. The blood serum of a patient having typhoid fever, for example, will not only agglutinate the typhoid germ but will also cause precipitation in broth in which typhoid bacilli have been grown and from which they have been filtered off. In some cases, as in anthrax, the tissues suspected of coming from animals having this disease may be extracted with physiological salt solution and brought in contact with serum from an animal which has been immunized against the particular disease. The development of a precipitate shows the presence of the specific organism in the tissue under investigation.

Cytolysins, Including Bacteriolysins and Hemolysins

It was first noted by Pfeiffer that the blood serum of animals may sometimes possess the property of dissolving or digesting bacteria. This observation was first made with the organism which causes Asiatic cholera, the *Vibrio choleræ*. It was found that when these organisms were

injected into the peritoneal cavity of a suitable animal, samples withdrawn at intervals and stained preparations made for microscopic observation, the bacteria were found at first to stain sharply and definitely, later to stain indefinitely, then as granules, and at last completely to disappear. It was then found possible to immunize animals against various disease-producing organisms and to study in the blood serum of such immunized animals the substances capable of destroying the organism. Such substances were named *bacteriolysins.* Later it was found that the injection of other kinds of foreign cells would lead to the development of corresponding antibodies. For example, if red blood corpuscles (carefully washed to remove serum) of a guinea pig be injected into the blood of a rabbit, the latter will finally acquire the property of dissolving or digesting the red blood cells of the guinea pig. This phenomenon may be observed in a test tube. If a suspension of guinea pig corpuscles in physiological salt solution be made and a drop of blood serum from a rabbit which has been immunized against such corpuscles be added to it, the opaque red suspension will change in the course of a few minutes or half an hour to a transparent red solution. Such an antibody capable of bringing about solution of red blood cells is termed an *hemolysin.* Other types of cells were also found to be capable of inciting the development of lytic substances. Antibodies of this general type capable of dissolving cells are termed *cytolysins.*

Constitution of Cytolysins.—Cytolysins are thermolabile, that is, when exposed to a temperature of 56° C. or above for a half hour, they lose their power to destroy cells. However, this power to destroy cells may be completely restored by adding to the antiserum some serum from *normal* blood, that is, serum from an animal which has never been immunized. It is evident, therefore, that a *cytolysin is in reality made up of two substances; one pro-*

duced as a result of the immunization and present only in the blood of immunized animals; the other a normal constituent of blood serum common to the blood of most, if not all, species of animals. The substance produced as a result of the immunization, that is, the specific antibody, has been termed the *amboceptor.* The substance present normally in the blood serum of most animals is termed the *complement. Cytolysin, therefore, is amboceptor plus complement.*

Experiment has shown that amboceptors are highly specific. They unite only with the kinds of cells instrumental in causing their production. It may be shown experimentally that whenever amboceptor comes in contact with its specific antigen, the two unite. There is, however, no visible change of any kind. If complement is then added, it in turn unites with the antigen and brings about the hemolysis. The amboceptor, in other words, is a specific substance which sensitizes bacteria or other cells to the action of a universally present complement. This may be shown by mixing a heated antiserum, that is, a serum containing cytolysin in which the complement has been destroyed by heat (leaving the amboceptor only) with suitable cells, allowing them to stand in contact for a time, then washing repeatedly (by means of the centrifuge) in physiological salt solution. Cells treated in this fashion brought into contact with serum containing complements will be rapidly destroyed. Neither the heated serum alone, containing amboceptor, nor the normal serum alone, containing complement, is able to destroy the cells, but when the cells are sensitized by the presence of amboceptors, the complement is capable of destroying them.

It is apparent that immunity to many diseases is due, at least in part, to the production of these specific amboceptors in the blood. Such amboceptors, for example, may be demonstrated in the blood serum of a person who has recov-

ered from typhoid fever. It is possible also to produce antisera containing specific bacteriolysins in the horse, for example, and to use such antisera in the treatment or prevention of disease. The serum used in the treatment of the disease cerebrospinal meningitis usually contains considerable amounts of bacteriolysin specific for the meningococcus, the organism which causes this disease.

Complement Fixation in the Diagnosis of Disease.—In certain diseases amboceptors specific for the causal microorganism are formed early. Their development does not necessarily mean the recovery or effective immunization of the patient. This is particularly true in certain chronic infections. For example, the disease glanders in the horse may and frequently does assume a chronic form which is not at all readily recognized by clinical examination. Whether or not the animal has the disease, however, may be determined by examining a sample of the blood for the presence or absence of the amboceptors specific for the glanders bacillus. If they are present it is usually considered as conclusive evidence that the animal has the disease glanders. This test, that is, hunting for specific amboceptors in the blood of suspected individuals, is termed the complement fixation test. Two groups of substances are necessary. The first is that group required for the specific reaction, and second, a group used as an indicator for the recognition of the reaction.

The materials of the first group are as follows:

1. *Suspension of specific organisms such as glanders bacillus (the antigen).*

2. *Serum from the animal, such as the horse, suspected of having the disease.*—If the disease is present this serum will contain amboceptor and complement. The latter is removed, however, before the test by heating. A positive test will mean the presence of an amboceptor specific for the glanders bacillus.

3. *Blood serum containing complement.*—This is usually obtained from the guinea pig. It is carefully titrated before use in order to determine the amount of complement present.

Suitable amounts of numbers one, two and three, that is, of the antigen, serum containing amboceptor, and serum containing complement are mixed. The amboceptor should at once unite with the specific organism, such as the glanders bacillus, and then the complement unite with this sensitized antigen. Theoretically one might expect to determine the presence of amboceptor then by examining such material microscopically. Practically, however, this does not prove to be feasible. The changes brought about by the complement in the bacteria may be in some cases so slight that they cannot be observed microscopically. It will be noted, however, that if amboceptor is present the complement will be used up or *fixed.* If amboceptor is not present the complement will not be fixed. To determine then, whether or not the amboceptor is present, some reagent is desirable which may be added to this mixture to determine whether or not the complement has disappeared. This reagent (the second group of materials) is prepared from the following:

4. *Red blood cells of some suitable animal such as sheep.*—These should be washed carefully and suspended in physiological salt solution.

5. *Serum from rabbit which has been immunized by repeated injection of sheep red blood corpuscles.*—This is heated to destroy any complement present. It should contain amboceptors specific for sheep red blood cells.

Number four and five are mixed. The amboceptor unites with the red blood cells, sensitizing them to complement. These sensitized cells may now be used as an indicator in solutions for the presence or absence of complement. This mixture of 4 and 5 is added to the first mixture, containing

1, 2, and 3. If no hemolysis occurs it is evident that the complement has been used up, therefore, that there is amboceptor present in the patient's serum and the disease is diagnosed as glanders. On the other hand, when the mixture of 4 and 5 is added to 1, 2 and 3 hemolysis will occur if the complement has not been fixed. This will occur if amboceptor is absent. The diagnosis then would be negative.

A somewhat similar method, usually termed the Wassermann test, is commonly used in the diagnosis of syphilis in

FIG. 61.—PHAGOCYTOSES. 1. Of bacilli. 2. Of cocci.

man. While theoretically many disease-producing organisms cause the development of amboceptors, their presence in the body is usually not used as a means of diagnosis with the exception of the diseases glanders, syphilis, Malta fever and gonorrhea.

OPSONINS AND PHAGOCYTOSIS

It has already been noted (page 278) that Metschnikoff first called attention to the fact that certain body cells, particularly certain types of white blood corpuscles are

capable of engulfing and destroying foreign cells such as bacteria which may gain access to the body. He conceived immunity, therefore, as being the process of training the white blood cells to engulf and destroy invading microorganisms. Later two English investigators, Wright and Douglas, showed that the white blood cells by themselves usually do not possess the ability to engulf and destroy microörganisms, but they require the presence of a serum constituent of antibody to which the name *opsonin* has been given. They showed that immunity in many diseases is due to the development of these opsonins which so change the bacteria that they become attractive or positively chemotactic for the white blood corpuscles.

Detection of Opsonin.—The fact that white blood corpuscles have the power of destroying bacteria may be shown by making injections of suitable bacteria into the blood stream of an animal, removing samples of blood at intervals and preparing stained mounts. By the use of an appropriate dye, the cytoplasm of the white blood corpuscles will stain very lightly, the nucleus of the cell deeply and any bacteria either on the inside or outside of the cell will also stain deeply. It is possible by microscopic observation in this fashion to count the number of bacteria which have been engulfed by a white blood cell. Study will show that the bacteria in the blood will at first be free, later and in larger proportions, they will be found inside of the white blood cells. Finally they will begin to break to pieces, become granular and finally completely disappear.

Similar observations may be made outside of the body. For this purpose it is necessary to secure suspensions of white blood corpuscles, bacteria and the serum to be tested. The white blood cells are secured by centrifuging blood which has been prevented from coagulating by the addition of sodium citrate. The red blood cells, having a higher specific gravity, settle to the bottom. The serum remains

on top. The white blood corpuscles are intermediate and will appear in large numbers in the surface layers of the sediment. The serum may be then pipetted away, the white blood cell layer removed by means of a small spoon or spatula, resuspended in physiological salt solution and centrifuged once or twice again to wash away all of the serum. The cells are then suspended in physiological salt solution for study.

A suspension of the bacteria to be studied is also prepared in physiological salt solution. A suitable dilution of the serum to be tested is also prepared. By means of a capillary pipette a small amount of the suspension of white blood cells, and equal amounts of the bacteria suspension and of the serum to be tested are drawn up, then blown out upon a glass slide and mixed. They are then drawn again into the pipette, the end sealed and placed in an incubator at 37½° C. for fifteen minutes. The mixture is then blown again onto a glass slide, spread, fixed and stained. In general there will be a direct relationship between the amount of opsonin specific for the organism used present in the serum and the number of bacteria engulfed on the average by white blood corpuscles. If bacteria and white blood cells alone are mixed without serum containing opsonin, very few if any of the bacteria will be engulfed.

Opsonic Index.—It is possible to compare the opsonin content of one individual and that of another by determination of what is termed the opsonic index. This is a number which represents roughly the ratio between the amount of opsonin contained in the two bloods. When white blood corpuscles, bacteria and serum from a patient, for example, are tested and the average number of bacteria engulfed per white blood cell determined, and the same process carried out with the same bacteria, white blood cells and serum from a normal individual, the ratio of the average number of bacteria engulfed as the result of the use of patient serum

with that of those engulfed when using normal serum, is termed the opsonic index. For example, if patient serum on the average should cause the white blood cells to engulf an average of ten bacteria, and the normal serum cause the white blood cells to engulf five bacteria, the opsonic index would be the ratio of ten to five.

The Presence of Opsonins in Antisera.—Certain types of antisera are prepared by injection of suitable animals with dead or living cultures of bacteria in an effort to secure a high concentration of opsonin specific for the disease in the blood of such an animal. Such sera are sometimes used in the treatment of disease. Most of the so-called antibacterial (not antitoxic) sera used in disease treatment contain opsonins and usually cytolysins as well.

Significance of Opsonins in Immunity.—It is probable that the body owes more of its resistance to disease to the presence of opsonin than to the presence of any other single antibody. Development of opsonin may be stimulated by the use of vaccines. A vaccine may be defined as a dead or attenuated culture of an organism injected into an animal or individual for the purpose of causing it to develop an active immunity. The immune substances formed are probably in large part cytolysin and opsonin. In some cases the term *vaccine* is restricted to living or attenuated bacteria, and for dead cultures injected in a similar fashion the word *bacterin* has been coined.

It is possible, following vaccination, to note increased resistance by studying the opsonic index of the vaccinated individual.

Hypersusceptibility or Anaphylaxis

In certain respects the phenomenon of anaphylaxis or hypersusceptibility is the converse of immunity. It is, nevertheless, very closely related to the latter phenomenon and should be discussed, in consequence, at this point.

It has long been known that certain substances, particularly certain proteins, are poisonous to certain individuals. There are some persons, for example, who cannot eat eggs without being poisoned thereby. The specific phenomenon, however, was not carefully investigated until after certain accidental observations were made upon the conduct of guinea pigs. In the study of the standardization of antitoxin attempts were made to use guinea pigs a second time as test animals, that is, animals which had received doses of antitoxin and toxin and had recovered were later tested out to determine whether or not they might be utilized a second time for standardization. It developed, however, that almost invariably a second injection of antitoxin proved fatal. This led to a study of the effect of injecting pure proteins into guinea pigs. It was found whenever a dilute solution of a protein is injected under the skin of a guinea pig or introduced into the blood stream, the animal then allowed to remain without subsequent injection for a period of two weeks or more, that a second injection made after the end of this period resulted in the development of a certain reaction which has been termed the *anaphylactic shock*. For example, a guinea pig may be injected with a small quantity of egg white. It will show no untoward reactions, will remain apparently healthy and vigorous, but when injected intraperitoneally or intravenously two weeks or more later with a similar solution of egg white there will be a rapid development of severe symptoms. The animal will show difficulty in respiration. It will urinate and defecate rapidly. It will usually scratch its nose with its front paws, thus showing evidence of difficulty in respiration. It may finally develop convulsions, fall over on its side and die within a few minutes after injection. What was, upon the first injection, a nonpoisonous substance is found upon a second injection to become a deadly poison for the guinea pig. An animal which has

been thus sensitized to the protein is said to be in a condition of *hypersusceptibility* or *anaphylaxis.*

The anaphylactic reaction is very specific. A guinea pig injected with one kind of protein does not become sensitized thereby against other kinds of protein.

Other species of animals than the guinea pig may be similarly sensitized by the *parenteral* introduction of protein, that is by an injection or introduction of protein by some means other than through the normal channel of the alimentary tract. The symptoms developed as a result of the second injection into these other animals may differ materially from those exhibited by the guinea pig.

Examples of Natural Anaphylaxis.—It was noted above that occasionally individuals may develop sensitiveness to the presence of particular proteins without any intentional preliminary sensitization. Such, for example, is the sensitization of individuals to the pollen of certain plants. In the United States in many localities the rag weed pollen during the months of August and September causes intense irritation of the mucous membrane of the respiratory tract of those individuals said to have the disease *hay fever.* Just how such individuals became sensitized, it is difficult to ascertain.

Certain individuals become sensitized to the proteins specific for particular animals. There are persons, for example, that cannot be in the vicinity of a horse or inhale the scurf from the skin of the horse without being subjected to an attack of asthma. In fact many types of asthma are undoubtedly due to the inhalation of particles of dust containing proteins or other substances to which the individual has in some way become sensitized. Individuals who have thus become sensitized to proteins will show the characteristic reaction in a variety of ways. If an extract of rag weed pollen be rubbed vigorously into the skin of a person subject to hay fever caused by this particular type of

pollen, a marked inflammation and swelling at this point will be noted. It will not have this effect if rubbed into the skin of a normal individual. In a similar way an extract from the hair or skin of the horse rubbed into the skin of a person subject to horse asthma will cause a decided local reaction.

Occasionally individuals are found who are subject to anaphylactic shock when injected with horse serum. Care must be used, therefore, in injecting diphtheria antitoxin and other antisera to make sure that the individual is not markedly anaphylactic toward this protein. In some instances the injection has been known to cause a temporary illness and is not infrequently followed by the development of a rash.

Desensitization.—If a guinea pig be sensitized by the injection of protein, and two weeks or more later a second injection of the same protein be made in a dose too small to cause fatal results, yet large enough to evoke some of the symptoms of the anaphylactic shock, the animal will for a time be temporarily desensitized. That is, after the anaphylactic shock has been evinced a third injection of the same protein, unless much larger, will not produce the anaphylactic symptoms. Use has been made of this principle in desensitizing individuals sensitive to particular proteins.

Utilization of the Anaphylactic Shock in Disease Diagnosis.—When certain bacteria, particularly organisms causing chronic infections such as glanders or tuberculosis, gain access to the body they apparently act in the same manner as a sensitizing dose of a protein. That is, as a result of their growth in the body the individual animal becomes highly sensitized to the organisms and the products of their growth. This fact is made use of in the recognition or diagnosis of such diseases. For example, a cow which has been infected with tuberculosis becomes hypersensitive to

the products of growth of the tubercle bacillus. Consequently it is possible by growing the tubercle bacilli in the laboratory in broth, killing the organisms by heat, filtering and concentrating the product to develop what is practically a glycerin extract of tubercle bacilli which, when injected into animals suspected of having tuberculosis, will determine the presence or absence of the disease by the reaction produced. If the injection is made subcutaneously, there will generally develop in from twelve to sixteen hours after injection a fever leading to a rise of one or more degrees in the temperature. If the injection is made into the skin there may be considerable local swelling developed. A purified tuberculin brought into contact with the mucous membrane covering the eye will cause decided inflammation. The material thus prepared for diagnosing tuberculosis has been termed *tuberculin*. A similar material prepared from glanders bacilli termed *mallein* has been extensively used in the diagnosis of glanders in the horse.

CHAPTER XXII

MICROÖRGANISMS OF THE BODY IN HEALTH AND DISEASE

Bacteria of the Body.—Many of the bacteria found associated with the bodies of plants and animals are saprophytic and are found in these locations by accident. Soil and water contain great numbers of living organisms and many are associated with dust particles in the air. Such organisms, except as they may lead to some confusion in studies of organisms normal to the body, are of no particular importance from the standpoint of health or disease.

The bodies of animals and many plants harbor what may be termed a normal flora. Many of the organisms composing this flora are commensals, that is, they live upon the waste products of the host and do not in any way cause disease. Some are even decidedly useful. It is probable, for example, that the digestion of cellulose in the alimentary tract of ruminants and herbivorous animals in general is due, at least in part, to the activity of certain kinds of bacteria. Still other organisms are true parasites, living in or on the tissues of the body. In a few instances these are known to be helpful. The bacteria, for example, which live in the roots of leguminous plants are truly parasitic, yet they are on the whole helpful and not injurious to the plant.

Many of the bacteria present in the body are capable of producing disease under certain conditions. Some kinds of organisms have very low degree of virulence or attacking power and under normal conditions are quite incapable of destroying or injuring living tissues. Other forms of bacteria which occasionally gain admission to the body are

highly virulent and in many cases can overcome resistance readily.

Certain species of cocci, particularly organisms belonging to the genus *Staphylococcus,* are abundant upon the skin. These bacteria, together with certain species of *Streptococcus,* are found frequently in the mouth and intestines. In the alimentary tract, particularly in the colon, a considerable number of species of bacteria grow normally. Most important and most common in this group is the organism known as the *Bacterium coli.* Under certain abnormal conditions the bacterial flora of the intestines may contain a great variety of other kinds of bacteria.

How Bacteria Gain Admission to the Tissues of the Body. Infection Atria.—Microörganisms capable of causing disease gain admission to the body in a variety of ways. In some cases they enter as a result of *traumata,* that is, through direct mechanical injury to the skin or mucous membranes. Bacteria, for example, frequently gain admission through wounds, giving rise to inflammation, pus production and sometimes generalized infection. A specialized type of traumatic infection is to be found in the bites of certain insects. The mosquito, for example, when infected from the blood of a person having malaria may later inject the organisms capable of causing this disease into another individual. In a few cases organisms may apparently gain access to the body through the *unbroken skin* or mucous membrane and produce disease. Certain of the molds, for example, capable of causing diseases such as ringworm and favus apparently do not require any injury to the skin in order to begin their growth. A very frequent channel of infection is the *alimentary tract.* Many diseases are contracted as a result of swallowing the causal microorganisms. Typhoid fever is a disease of this type. In certain cases the *respiratory tract* constitutes the infection atrium. One may inhale, for example, the organism capable of causing pneumonic plague or other respiratory

diseases. Finally, certain deseases are transmitted usually through the *genito-urinary tract.* Such, for example, are certain of the venereal diseases, particularly gonorrhea and syphilis in man and dourine in the horse.

How Bacteria Leave the Body.—Inasmuch as most disease-producing bacteria do not multiply outside the body of the man or animal in which they live or produce disease, it is important to know the channels through which these microörganisms may leave the body. In some cases the organisms are found in the pus or exudates from the wounds or surface lesions of the body. In other cases they may be found in mucous secretions of the nose and respiratory tract, in still other cases in the saliva. The organism causing diphtheria, for example, is usually present in the throat and mouth. In many diseases the causal microörganisms are excreted with the feces. This is particularly true of the so-called intestinal diseases such as typhoid, paratyphoid, Asiatic cholera and dysentery. Occasionally the organisms may be excreted in the urine. This occurs occasionally in typhoid fever. Insects are sometimes responsible for withdrawal of organisms from the body when they suck up the blood. This occurs in yellow fever, malaria and some other diseases.

Bacteria causing plant diseases frequently are not released from the injured tissues until after the death and disintegration of the tissue. In other cases exudates may form and the organisms escape to surface. In still other cases apparently the disease organism is transmitted by sucking insects as in the curly leaf of the sugar beet.

The Types of Diseases Produced by Microörganisms.—In the animal body the microörganisms sometimes invade the blood stream, growing in the blood in all parts of the body. Diseases of this type are termed *bacteremias.* A bacteremia caused by entrance into the blood of an organism usually responsible for pus production and inflammation (such as *Streptococcus* or *Staphylococcus*) is frequently

termed a *septicemia.* Some authors use the term septicemia and bacteremia as entirely synonymous. A *toxemia* is a disease such as diphtheria or tetanus in which the causal microörganism remains more or less localized in or on the tissue where it produces a poison or toxin which enters the circulation and causes injury to the various body tissues. In some diseases the microörganisms tend to remain localized and produce marked inflammation without any generalized symptoms. Infections of this type are sometimes referred to as *phlogistic.* A *sapremia* is a disease caused by the absorption of poisonous putrefactive products from decaying or necrotic tissue. For example, the retention of the placenta or afterbirth in a cow after parturition and its subsequent decay or decomposition in the uterus may lead to the development of a sapremia.

Diseases are sometimes named after the causal organism. A disease caused by a *Bacterium,* for example, might be termed a *bacteriosis,* one caused by a *Spirillum* a *spirillosis,* and one caused by a *Spirochæta* a *spirochætosis.*

Diseases of plants produced by bacteria are sometimes known as galls or tumors, as for example, crown gall of the the apple; blights, such as pear blight; leaf spots; rots, such as the blackrot of cabbage; and wilts, such as the wilt of cucumber or sweet corn.

The infectious diseases, that is, the diseases caused by microörganisms, may be divided into two general groups; those in which the causal agency is definitely known, and those in which it is not definitely described. Under the first heading we have diseases which may be caused by bacteria, by yeasts, by molds and by protozoa. Under the second heading there are diseases caused by filterable viruses, that is, microörganisms apparently so small that, at least in certain phases of their life history, they can pass through the porcelain filters. In still other cases the causal organism has completely eluded the search of the bacteriologist thus far.

CHAPTER XXIII

NONSPECIFIC INFLAMMATION AND SUPPURATION—THE GENERA STREPTOCOCCUS AND STAPHYLOCOCCUS

Inflammation.—Inflammation is an abnormal condition of a tissue usually characterized by excessive redness, by pain, by swelling and usually by fever. The redness is due to the engorgement of the capillaries with blood, the swelling to the extrusion from the blood vessels of blood serum or plasma and the pain or pressure upon nerve endings. Inflammation may be produced in a variety of ways in the bodies of man and animals. It sometimes results from physical injuries. A burn or excessive exposure of the skin to bright light will lead to inflammation. The injection of certain chemicals will likewise product inflammation. There are also many species of bacteria which when growing in the tissues of the body will bring about inflammation. These bacteria probably bring about the irritation necessary to cause inflammation because of their injurious effect upon the cells either directly or through the formation of various poisonous substances.

Inflammation may be regarded as a protective device on the part of the body. When microörganisms gain access to tissues and give rise to inflammation, the changes incident to this process may prevent them from spreading to other parts of the body. The manner in which this is accomplished will be discussed below.

Suppuration.—Suppuration or pus production occurs when certain organisms are growing in tissues and causing localized inflammation. For example, when the so-called *pyogenic* or pus-producing bacteria in some way penetrate

the skin frequently they find conditions favorable for development. They have optimum moisture, optimum food conditions and optimum temperature for their rapid growth. They can be stopped only by specific defensive devices on the part of the body. As a result of their growth and of the irritation of tissues due to the resultant injury, the blood capillaries expand and become engorged. Serum is poured out into the tissues and passes into the wound. This serum ordinarily contains antibodies, particularly opsonins. As a result of the union of opsonin with the bacteria, the white blood cells are also attracted to the area involved. These white blood cells have the power of leaving the blood stream, passing out through the capillary walls, and soon come to lie in great numbers around the injured area. They are so tightly packed together in many cases that they constitute what is termed a *pyogenic membrane*, capable in most instances of effectually preventing further spread of the microörganisms through the tissues. The opsonized bacteria are then more or less rapidly destroyed by the white blood corpuscles. The blood serum and the white blood cells together with some bacteria and disintegrated tissue cells constitute *pus*, and a wound producing pus is said to *suppurate*. Eventually the microöganisms are usually disposed of and healing occurs as the result of the development of new tissue. It is evident that suppuration is one manifestation of an inflammation.

Nonspecific Inflammation.—As noted above, many kinds of bacteria are capable of producing inflammation. In some cases these bacteria are capable of causing the so-called specific diseases, such as the typhoid bacillus, the pneumococcus (producing pneumonia), or the bacillus of tuberculosis. In other cases inflammations are produced by what are termed the *nonspecific, pyogenic cocci*, that is microörganisms usually not responsible for the so-called specific infectious diseases, but capable of causing a great

variety of inflammations, the type depending upon the conditions governing infection and the degree of resistance of the individual.

An inflammation of any organ of the body is usually named by adding *"itis"* to the root of the name of the organ concerned. Examples of such inflammation are tonsilitis (inflammation of the tonsils); appendicitis (inflammation of the appendix), etc.

Three organisms are most commonly responsible for the nonspecific inflammations. These are the *Streptococcus*

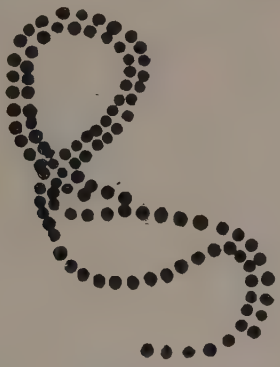

FIG. 62.—STREPTOCOCCUS PYOGENES.

pyogenes, the *Staphylococcus aureus,* and the *Staphylococcus albus.* Many other species have been described but are much less important. They differ only in minor respects from the organisms mentioned. These forms, therefore, are the only ones which will be discussed.

STREPTOCOCCUS PYOGENES

Synonym.—*Streptococcus erysipelatis.*

This organism probably should be regarded as a group of closely related species rather than as a single species. The classification of the various species, however, has not been satisfactorily worked out and the differences in disease pro-

duction or pathogenicity need not be emphasized here. It may be noted that *Streptococcus pyogenes* is morphologically very much like the *Streptococcus lacticus* usually associated with the souring of milk.

Morphology.—*Streptococcus pyogenes* is a coccus about one micron in diameter, practically always occurring in chains, sometimes short and sometimes long. It is nonmotile, does not produce spores, is easily stained and is Gram-positive. Some types produce capsules, although most of the more highly pathogenic forms are capsule-free.

Distribution.—Nonvirulent strains of *Streptococcus pyogenes* are quite common upon the surface of the skin and particularly in the mouth and on the mucous membrane. Virulent strains have been isolated from a great variety of inflammatory conditions.

Culture.—This organism grows fairly readily upon most of the ordinary culture media but not particularly luxuriantly. An agar slant culture, for example, develops in the form of minute colonies, rarely larger than a pin head, at first transparent and almost dewdroplike; later they may become somewhat more opaque. In general they grow particularly well upon coagulated blood serum, but not upon potato. In broth the medium is sometimes clouded as a result of the formation of numerous short chains, in other cases it remains clear, the organism evidently growing in the form of long tangled threads as a sediment in the bottom of the tube.

Physiology.—This organism is aërobic and facultative anaërobic. It grows best at blood heat but growth will usually take place also at room temperature. No pigment is produced and no coagulating or proteolytic enzyme. No gas is produced from any of the sugars, though acid is formed by most strains from many sugars, particularly from dextrose and lactose. Efforts, in part successful, have been made to classify the various strains of streptococci,

using acid production in various sugars as a basis for this classification.

Pathogenesis.—Various strains of *Streptococcus pyogenes* show very great differences in their ability to produce infection. Some strains are rleatively nonvirulent, others are highly virulent. Some when inoculated into the body tend to grow best in the blood stream, others grow in the joints or in some particular organ of the body. In general the disease produced is a more or less diffused inflammation. In general infections with *Streptococcus pyogenes* are somewhat more serious than those with staphylococci. Apparently it is somewhat more difficult for the body to rid itself of these organisms than of the other nonspecific pyogenic types.

Focal Infections.—*Streptococcus pyogenes* when growing in the body is apt to develop in some particular tissue or organ producing in it either an acute or chronic inflammation. As will be noted below, organisms from such chronic inflammations not infrequently pass to other parts of the body, being carried there by the blood stream. A few of the more important focal infections will be noted briefly.

Streptococci not infrequently produce chronic inflammation of the tonsils (*tonsilitis*). From these they may spread to neighboring lymph glands causing their enlargement or inflammation, sometimes resulting in the condition known as *quinsy*. Chronic tonsilitis (as is true in chronic inflammation of any part of the body) usually leads to the formation of more or less cicatricial or scar tissue, the tonsils becoming hardened, and local areas of inflammation persist. Occasionally the organisms pass to the middle ear, probably by way of the Eustachian tube, causing the condition known as *otitis media*. From this location the organism may also occasionally pass to the interior of the mastoid bone (or rather the mastoid process of the temporal bone)

lying just behind the ear, causing the condition known as *mastoiditis.* The latter condition is particularly dangerous because of the possibility eventually of the organism passing through the bony inner wall of the mastoid, gaining access to the brain cavity and there causing inflammation of the covering membranes of the brain and spinal cord or *meningitis.* Surgical intervention is sometimes necessary.

Organisms of this type may also cause ulceration of teeth and in improperly filled teeth the organisms may pass through the root, gain access to the gums and cause chronic inflammation (or pyorrhea) about the roots of the teeth, in some cases leading to a more diffused inflammation usually termed *gingivitis* or inflammation of the gum. The mucous membranes lining the nose may also be inflamed by organisms belonging to this group, giving rise to the condition known as *rhinitis* or more commonly as a *cold.* From these membranes the organism sometimes passes by means of the connecting canals into the sinuses of the head, that is, into those cavities in the bones lined by mucous membranes. Among the more important of these are the frontal sinuses. This condition is known as a *sinusitis.* Inflammation of the udder sometimes occurs in cattle as a result of infection with organisms of this general type, producing the condition known as *mastitis* or *garget. Erysipelas* in man apparently is due to the growth of this organism in subcutaneous tissues producing extensive or general inflammation.

In some cases a focal infection with a highly virulent organism may lead to the passage of the organism to the blood stream and the development of *septicemia* (or "blood poisoning"). Certain types of *pneumonia,* particularly many following influenza, are due to infection of the lungs from highly virulent strains of streptococci. Streptococcus infection is not uncommon in lower animals. Such, for example, is the so-called *navel ill* of the colt. The organism enters through the umbilicus soon after birth. Wound

infection and suppuration in both man and animals are sometimes caused by streptococci, although in most cases staphylococci are more abundant.

As a result of focal infections in various parts of the body, more particularly in tonsils, about the teeth, and in the sinuses, *Streptococcus pyogenes* may gain entrance to lymph channels or the blood stream and localize in other parts of the body. Most cases of *articular rheumatism,* for example, are of this nature, the organism localizing and growing in the membranes about the joints. Another secondary effect following tonsilitis and similar infections is *endocarditis,* that is, inflammation of the lining membranes of the heart, including the membrane which covers the various heart valves. In a certain proportion of cases the latter leads to a deposition of fibrin upon the heart valves, later the development of scar tissue and the warping of the heart valves out of shape, resulting in leakage through the valves. In most cases the organisms are finally eliminated but the defective heart valve, of course, cannot be repaired. This is one of the most common causes of heart trouble. In some cases the fibrin collecting upon the heart valves is considerable in amount. It may form small cauliflowerlike masses. Portions of this material occasionally break away and pass into the blood stream. Such a particle is termed an embolus and when it reaches a blood vessel too small for its passage it may obstruct the flow of blood. When this occurs in an end artery it may entirely cut off the blood supply from that portion of the body. If this should occur in the brain, for example, it would lead to *paralysis.*

Immunity.—Resistance to infection by streptococci seems to be due principally to the activity of opsonins and white blood cells. The problem is rendered complex by the fact that there are many strains, varieties or perhaps even species of streptococci and the opsonins or immune bodies

for each are so specific that they will not immunize against other strains. Immunization against streptococcus infections by the use of vaccine, consisting of suspensions of killed streptococci, has been frequently attempted. While some favorable results have been reported, the results on the whole do not appear to be particularly encouraging unless great care is taken that the organisms injected are the ones against which immunity is actually desired. So-called *autogenic* vaccines have been used with some degree of success in chronic infections. In chronic erysipelas, for example, there are reports of favorable results secured by injecting dead cultures of the type of organism causing the infection in the particular individual to be protected. Streptococci are common constituents of many of the so-called influenza vaccines, inasmuch as the streptococci have been found in many instances to produce the post-influenza pneumonias.

Vaccines against streptococcus infections have been prepared in a variety of ways. Todd made such a vaccine by first growing the streptococci on blood serum for twenty-four hours, and inoculating from these cultures into large flasks containing ten per cent serum broth. These were incubated for one month. Six per cent of sterile glycerin is added and this material concentrated at 60° C. for two days over unslaked lime. It is evident that the thick paste thus secured is made up of the products of autolysis of the bacteria, as well as the growth products and unchanged serum broth. It is diluted before use as a vaccine. Other investigators have prepared bacterial extracts by shaking broth cultures either with urea or galactose in twenty-five per cent solution for several days. The high concentration of the solution tends to destroy the organisms, and the shaking to disintegrate them. The vaccine is thereby rendered sterile without heat, and the antigen, therefore, is not altered.

In some cases the streptococci are grown in broth in large quantities, and separated by centrifugation. An apparatus working on the principle of a cream separator has been devised and used for this purpose.

A hemolytic toxin, streptolysin, has been demonstrated for some strains of *Streptococcus pyogenes*, and for this an antitoxin has been produced. However the antitoxin has not been shown to have any curative or prophylactic effect. It is not probable that the disease-producing power of the organism can be accounted for on the basis of toxin produced. So-called endotoxins are developed both by virulent and by non-virulent strains. Bacteriolysins are probably not important. Immunity is largely to be ascribed to the activity of opsonins.

Antisera prepared from horses by injecting first with dead and then with living cultures of streptococci until a high degree of immunity has been obtained, have been claimed to be successful by some users. The results have not, however, in general proved encouraging. In general it may be stated that no very satisfactory method of immunizing against *Streptococcus pyogenes* has been developed.

Staphylococcus aureus and Staphylococcus albus

Synonyms.—*Staphylococcus pyogenes aureus* and *Staphylococcus pyogenes albus, Micrococcus aureus* and *Micrococcus albus, Aurococcus aureus* and *Albococcus albus.*

Morphology and Staining Characters.—These organisms are considered together inasmuch as they are found in the same general situations, are very closely related, differing from each other only in minor characteristics such as pigment production. These bacteria are spherical, occurring in irregular masses like grape clusters. The cells are usually 0.7μ–0.9μ in diameter, occasionally larger. They are non-

motile, do not produce spores, stain readily with the ordinary aniline dyes and are Gram-positive.

Distribution.—The staphylococci are quite common upon the skin and hair in man and animals, likewise in the nose and mouth and occasionally in the feces. They are not uncommon in water, especially when this has been contaminated with sewage. They are present in many cases of local infection accompanied by pus production.

Cultural Characters.—These bacteria may frequently be secured in pure culture directly from the pus of suppurating wounds, boils or abscesses. The bacteria grow readily upon the ordinary culture media. In gelatin a slow liquefaction occurs. The two species may be differentiated from each other in general by the color produced upon media. When grown upon potato, for example, *Staphylococcus aureus* produces a bright orange pigment while the *Staphylococcus albus* is white. Growth in milk is followed by the production of some acid and ultimate digestion of the curd.

Physiology.—Small amounts of acid are produced from some sugars, but no gas. Nitrates are reduced to nitrites. Enzymes capable of digesting casein and gelatin have been demonstrated.

Pathogenesis.—The staphylococci in general produce infections such as abscesses, carbuncles, boils, acne, furuncles in man, wound infection, poll-evil and fistula in the horse, and similar lesions in other animals. Inasmuch as these bacteria are normally present upon the surface of the skin, they generally invade the tissues whenever there is a break in the skin, that is, through any injury. They are therefore, the common causes of wound infection or suppuration in wounds. A *boil* results from the entrance of organisms of this group under the skin, where they grow (providing the individual is not sufficiently immune) and destroy a certain amount of tissue. A reaction or inflammatory condition is set up immediately about the injured

portion. The pyogenic membrane previously described is formed and the bacteria are prevented from spreading from the initial source of infection. The portion of the tissue killed by the bacteria gradually softens and is discharged through the surface, that is, the boil is said to "come to a head." The purulent discharge which follows is a mixture of white blood cells, serum, bacteria and disintegrated tissue. When the bacteria have been cleansed from the

FIG. 63.—STAPHYLOCOCCUS AUREUS.

injured area, cicatricial tissue or scar tissue grows into the wound, replacing the tissue destroyed by the microörganism.

A somewhat similar condition, frequently becoming chronic, is the *fistula* on the withers or shoulders of a horse. The organisms have gained access through the skin as a result of a bruise or injury, grow between the muscles and layers of fascia, producing severe inflammation. In chronic fistula the area is more or less surrounded by a growth of scar tissue. *Poll-evil* is a somewhat similar affection on the top of the poll or head of the horse.

Immunity.—As in infection with *Streptococcus*, immunity, when developed, is probably due to the presence of sufficient quantities of opsonin. The popular conception that boils are beneficial because they drain impurities from the blood is fallacious. A person is subject to boils primarily

because of a deficiency in immunizing substances, probably in large part opsonin. Occasionally apparently the presence of bacteria in a wound or in a boil is not sufficient to incite the individual to the production of immune substances or antibodies such as opsonin. In such instances, boils may recur at frequent intervals and the individual is said to suffer from *furunculosis*. Under such conditions it has been found possible to use successfully vaccines prepared from the organism causing the trouble in the particular individual. These are prepared by growing the bacteria on slanted agar cultures, suspending in physiological salt solution, destroying by heat. Subcutaneous injection of such a vaccine tends to incite the tissues to the production of suitable antibodies.

CHAPTER XXIV

PNEUMONIA, MENINGITIS AND GONORRHEA—THE SPECIFIC PYOGENIC COCCI—THE GENERA DIPLOCOCCUS AND NEISSERIA

The organisms belonging to the genera *Diplococcus* and *Neisseria* include for the most part pathogenic, or at least parasitic bacteria, spherical in shape, whose cells occur generally in pairs.

The organisms may be differentiated by use of the Gram's stain. The members of the genus *Diplococcus* are Gram-positive, those of the *Neisseria* Gram-negative. The organisms belonging in this group which are pathogenic and of economic importance are three in number: the *Diplococcus pneumoniæ*, causing pneumonia; the *Neisseria meningitidis*, causing infectious cerebrospinal meningitis in man, and the *Neisseria gonorrhœæ*, causing the disease gonorrhea in man.

These organisms include the most common forms of the so-called specific pyogenic cocci, that is, those cocci responsible for inflammatory changes in connection with specific diseases.

Diplococcus pneumoniæ

Synonyms.—*Streptococcus pneumoniæ, Diplococcus lanceolatus.*

The organism is usually termed the pneumococcus.

The term pneumonia literally designates an inflammation of the lungs. This may be caused by any one of several species of bacteria. In the great majority of cases the pneumococcus is the cause and is ordinarily spoken of or referred to as the cause of specific infectious pneumonia.

Distribution.—Several distinct types, races or varieties of pneumococcus have been described. Some of these are not uncommon in the normal mouth and throat. Others are usually associated with disease production or are present in the mouths and throats of those asscciated with those who are diseased. Within the same type there are apparently considerable variations in virulence. Probably in no disease do we have a better illustration of the fact that production of disease is due to lack of immunity on the part of the individual attacked or to high virulence on the part of the attacking organism. In other words, the ability to produce disease always depends upon the relative virulence of the organism and the resisting power of the individual.

Morphology.—The *Diplococcus pneumoniæ* usually occurs in pairs, more rarely in chains with few elements. The cells are sometimes spherical, but are most frequently somewhat flattened at the point of contact and with the opposite side somewhat elongated and pointed. This lance-shaped appearance has given rise to the name *Diplococcus lanceolatus*. Capsules may usually be readily demonstrated in sputum or in body fluids. The organism stains readily and is Gram-positive.

Cultural Characters.—The pneumococcus is most readily isolated upon blood agar, that is, agar which has been melted and has had added to it a small amount of fresh blood which has been drawn under aseptic precautions to insure its sterility. If culture is desired from sputum where other organisms are apt to be present, it is usually necessary first to inoculate a suitable laboratory animal, such as a mouse. The colonies are never luxuriant, the organisms developing as discrete, transparent, dewdroplike colonies upon the surface. Broth is clouded and milk may be somewhat acidified.

Physiology.—The pneumococcus grows but little or not at all at temperatures lower than blood heat. Acids are

produced from certain carbohydrates. Cultures lose their viability relatively quickly. When the organism is suspended in broth or physiological salt solution it readily undergoes autolysis or self-digestion.

Pathogenesis.—The pneumococcus was first described as an organism present in sputum in healthy individuals, which when injected into a mouse, produced a quickly fatal type of septicemia. Later it was found quite consistently associated with acute infectious pneumonia in man and in certain animals, particularly the horse. In pneumonia the organisms are present in the lung tissue causing severe local inflammation. The blood vessels become engorged and

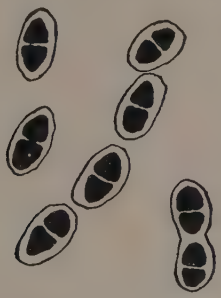

FIG. 64.—DIPLOCOCCUS PNEUMONIAE.

blood plasma is poured out into the air sacs or alveoli. The fibrinogen is converted into fibrin and the alveoli become filled with fibrin clots. Lung tissue which has been changed in this fashion is no longer spongy and capable of floating upon water, but is dense and when placed in water sinks to the bottom. Such lung tissue is said to have undergone hepatization, that is, it has become liverlike in consistency. In some cases there is more or less diffused bleeding into the tissues and they become red. Such a condition is known as *red hepatization.* When white blood cells are present in great numbers, the lungs are said to have undergone *gray hepatization.* The organisms may also gain entrance to the

blood stream, causing septicemia. In fact, septicemia is apparently, in many cases at least, the immediate cause of death from pneumonia. When the body defenses gain the upper hand, the bacteria may be eliminated and the lung tissues undergo resolution. The clotted contents of the alveoli undergo partial or complete autolytic digestion and the material passes to the mouth or is resorbed into the blood.

Bacteriological Diagnosis.—The *Diplococcus pneumoniæ* is usually recognized in sputum, when stained by Gram's method, as a capsulated diplococcus, Gram-positive. Inasmuch as several distinct types of pneumococci have been described, each capable of causing pneumonia in man and perhaps in animals, it is sometimes necessary to differentiate the types by the use of the agglutination test. Antisera, capable of conferring immunity and having curative properties, have been prepared for certain types of pneumococci, but not for others. It is of advantage, therefore, for a physician to know what type of pneumococcus is causing the disease in a particular case. This may be ascertained by securing a pure culture of the organism causing the particular infection and testing it against known antisera containing agglutinins. The pneumococci are usually divided into four types. Types 1, 2 and 3 are most commonly present in diseases and are relatively distinct and easily recognized. Type 4 includes forms not included in types 1, 2 and 3. The test is frequently carried out as follows: a bit of sputum from the patient is injected intraperitoneally into a mouse. At the end of a few hours the animal is killed and the peritoneal cavity washed out with physiological salt solution. The pneumococci will have multiplied rapidly and by this means a milky suspension of the organism may be secured in relatively pure culture. Portions of this suspension are then tested against sera known to agglutinate respectively types 1, 2 and 3.

Immunity.—The problem of immunization against pneumonia is complicated by the variety of types of pneumococci and other organisms causing the disease. It is also complicated by the marked variation which pneumococci apparently may show in virulence and by the great differences which may occur in resistance or immunity to the disease. Specific antisera have been prepared for certain types by injecting horses or other suitable animals, first with killed and finally with living cultures of pneumococci. The use of a potent antiserum against the right type of microörganism has proved markedly advantageous. Difficulty in typing organisms and in the preparation of potent antisera has interfered with its widespread use.

Pneumococci together with streptococci have generally been included in considerable numbers in the vaccines used to prevent influenza or rather to prevent the development of secondary complications following attacks of influenza. The evidence concerning the value of these vaccines is very conflicting.

Immunity against pneumonia apparently is short-lived. While a person convalescing from the disease may show evidence of a high degree of acquired immunity, this usually does not persist for a very long period.

Neisseria gonorrhœæ

Synonyms.—*Micrococcus gonorrhœæ, Diplococcus gonorrhœæ.*

The causal name usually applied is gonococcus, occasionally diplococcus of Neisser.

The disease produced is gonorrhea in man, not transmissible to animals.

Morphology.—Stained mounts of gonorrheal pus show the Gram-negative, coffee-bean-shaped diplococci, lying generally in pairs inside the polymorphonuclear leucocytes. In culture there is little or no tendency to chain formation,

the cells being arranged in irregular masses or in pairs. The gonococcus stains readily with the ordinary aniline dyes. The fact that it is Gram-negative facilitates differential diagnosis between infections with gonococcus and the other pus-producing bacteria.

Culture.—The gonococcus is usually secured by smearing serum agar in plates with gonorrheal pus. The colonies which develop resemble somewhat those of streptococci, being small, well-separated and transparent. Care must be used to maintain a medium that has a moist surface and the right hydrogen ion concentration, best that of the blood. After a few transfers the organism develops more luxuriantly on artificial media.

Physiology.—The gonococcus is aërobic, easily destroyed by drying, and in the laboratory rapidly undergoes autolysis, making it necessary to transfer cultures every two or three days when they are kept at blood heat. The organism remains viable in culture media for a somewhat longer period of time in the ice chest.

Pathogenesis.—Gonorrheal infection of the membranes of the eye sometimes occurs at birth, leading to the development of the so-called *gonorrheal ophthalmia*, or *ophthalmia neonatorum*. In some localities physicians and midwives are required to drop solutions containing suitable silver salts into the eyes of newborn children to prevent the development of this disease.

Gonorrhea is usually regarded as the most important of the so-called venereal diseases. In the male it is first an acute inflammation of the urethra which extends in many cases to the prostate gland. Chronic inflammation of this gland may lead to the formation of scar tissue and a decrease in the size of the urethral tube, producing the so-called *stricture*. In the early stages the disease is characterized by considerable quantities of purulent discharge. When it becomes chronic, as it may in prostatic infection,

the discharge changes to a more or less viscous serum. The gonococci occasionally invade the urinary bladder, producing an acute *cystitis*. They may also pass along the vas deferens to the seminal vesicles and finally to the epididymis, causing *epididymitis* and *orchitis*. The disease is peculiarly difficult to treat because after the first occurrence of urethritis, the organism frequently invades the neighboring glands, such as the prostate, and local applications of disinfectants do not reach them. In a considerable percentage of cases, therefore, the disease becomes chronic. In metastatic infection from cases showing prostatitis or vesiculitis, the organisms may localize in the joints producing gonorrheal rheumatism. More rarely there may be involvement of the heart valves.

In the female gonorrhea is usually primarily a vaginitis followed by involvement of the uterus and not infrequently of the Fallopian tubes. The acute inflammation here may give rise to the condition known as "pus tubes" (*pyosalpingitis*) in which there is danger of the tubes bursting and producing peritonitis. The urethra is frequently also involved in the disease.

Because of its wide distribution, the variety of the symptoms and the seriousness of the after effects, such as rheumatism, sterility, pyosalpingitis requiring surgical intervention, etc., the disease probably is to be regarded as the most important of the venereal infections.

Bacteriological Diagnosis.—As noted above, the disease may frequently be recognized by the identification of the Gram-negative, coffee-bean-shaped diplococci present in the pus. The complement fixation test has also been used to detect the presence of the organism where there are no apparent or evident symptoms of the disease, particularly in determining when a cure has been effected.

Immunity.—Gonorrhea is a disease from which spontaneous cure occurs but slowly, the individual frequently

retaining the organisms for months or for years. Apparently in some cases the organisms lie dormant or latent and produce symptoms not sufficiently acute to be noticed for long periods of time. Under certain conditions the disease may flare up again.

No antisera have been prepared which give relief or effect a cure in this disease. It has been claimed that vaccines have been used with some success in immunizing those who have the disease in chronic form.

Neisseria meningitidis

Synonyms.—*Diplococcus meningitidis, Diplococcus intracellularis, Micrococcus meningitidis.* The organism is frequently known by its casual name, meningococcus.

The disease produced, acute contagious cerebrospinal meningitis, is characteristic of man, although it may be transmitted by experimental inoculations to some animals, particularly to the monkey.

Morphology.—The meningococcus in the body exudates usually appears as a diplococcus, or occasionally in groups of four. In culture media, the organism is usually about 1μ in diameter and commonly occurs in pairs, rarely in short chains. Capsules are not produced. The organisms stain readily with the usual aniline dyes and are Gram-negative. Inasmuch as there are other organisms, such as streptococci and the pneumococcus which occasionally cause meningitis, the latter fact is important in differential diagnosis.

The organism may frequently be secured in pure culture by making a lumbar puncture with a sterile hypodermic needle and transferring directly to a suitable medium. The medium must be carefully prepared, have the correct acidity and usually it is necessary, upon first culture at least, to add blood serum. Upon this medium white discrete colonies develop. The organism undergoes autolysis or self-digestion so readily that frequent transfers must be made to keep it

viable except under the most favorable of conditions. Growth does not occur readily upon most of the ordinary culture media.

Physiology.—The meningococcus is killed readily by drying. Small amounts of acid are produced from dextrose; milk is not changed and gelatin is not digested.

Pathogenesis.—The meningococcus apparently gains access to the body through the respiratory tract. It finds in many individuals a suitable site for growth in the nasopharynx, that is, the back portion of the nasal cavity just above the pharynx. From this point in a rather uncertain proportion of individuals the organism finds its way, per-

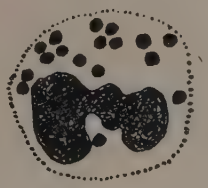

FIG. 65.—NEISSERIA MENINGITIDIS.

haps through the lymph channels or through the blood stream, to the central nervous system, where it grows upon and in the meninges (the membranes which cover the brain and the spinal cord). This is followed by the usual evidences of inflammation. The clear cerebrospinal fluid becomes clouded with white blood cells, that is, it becomes more or less purulent. A mount of this material secured by means of a lumbar puncture, that is, by the insertion of a hypodermic needle between the lumbar vertebræ into the spinal cavity, shows more or less numerous Gram-negative diplococci frequently lying within the white blood corpuscles. The disease is not infrequently accompanied by a septicemia, that is, the meningococci gain access to the blood stream and grow rather generally through the body.

Bacteriological Diagnosis.—It has already been noted that the disease produced by the meningococcus is differentiated from the other types of meningitis by identifying the organisms by means of lumbar puncture. They may usually be identified in a Gram-stained preparation. They may also be grown upon suitable culture media.

At least two relatively distinct types of meningococci have been described, some investigators insisting that there are even more, perhaps four. This is of importance because antisera prepared against one type does not appear to be particularly efficacious against another type. It is advantageous in some instances to determine the type of meningococcus causing the disease in the particular individual. Pure cultures may be secured and tested by the agglutination reaction against serum specific for each of the meningococcus types.

Immunity.—The problem of immunization against meningococcus is complicated, as is the case with the pneumococcus, by the fact that there are two or more distinct varieties. Antisera containing antibodies specific for the disease have been used with a marked degree of success. The antisera are prepared by injecting dead and, later, living cultures of meningococci into a suitable animal, such as the horse. When a high degree of immunity has been secured the horse is bled, the blood serum secured and filtered. This is usually injected intraspinally, that is cerebrospinal fluid is removed and a sufficient amount of the antiserum introduced. This comes in immediate contact with the microörganisms growing on the meninges.

CHAPTER XXV

THE COLON-TYPHOID SERIES OF BACTERIA
THE GENERA BACTERIUM AND PROTEUS

This group of organisms is frequently termed the *intestinal group* because many, although not all, of the organisms belonging to it are of intestinal origin, or find the best conditions for growth in the intestines of man or higher animals and sometimes of birds or fishes. The group is important because belonging to it are several organisms capable of causing disease in man and in animals, and because the detection of certain bacteria belonging to it constitute the most satisfactory method for the detection of pollution in water.

The genera *Bacterium* and *Proteus,* that is, the organisms belonging to the intestinal group of bacteria are rod-shaped, Gram-negative, aërobic or facultative forms which do not produce spores. Some species are motile, others nonmotile, some produce capsules, others do not. Frequently there is considerable power of fermentation of carbohydrates developed.

The genera *Bacterium* and *Proteus* are very closely related. The organisms belonging to the genus *Proteus* in general liquefy gelatin more or less rapidly, while only a few members of the genus *Bacterium* are capable of liquefying gelatin. These may be differentiated from members of the genus *Proteus* by the fact that the latter are never able to produce gas from the sugar lactose.

The genus *Bacterium* is much more important than the genus *Proteus* in the production of disease, and attention will, therefore, be concentrated upon the former. Certain

species of Bacterium (such as the *Bacterium lactis viscosum* producing ropy milk) will not be discussed in this section as they do not produce disease and are not of particular pathologic significance.

The true intestinal bacteria belonging to the genus *Bacterium* are divided usually into three principal sub-groups, the separation being made upon the basis of the ability to produce acid and gas in the sugars lactose and glucose. The bacteria which can produce acid and gas from both lactose and glucose belong to the *first* or *colon subgroup*. Those which can produce acid and gas from glucose, but not from lactose, belong to the *intermediate* or *enteritidis subgroup*. Those which produce no gas or acid from lactose, no gas from glucose, and may be either positive or negative as to acid production from glucose, belong to the *third* or *typhoid-dysentery* subgroup.

Each of these subgroups has been differentiated into a large number of species—about thirty altogether being well-characterized and recognized in the literature. It will be noted that in the differentiation of the subgroups the first shows high fermentative power and for the most part the organisms belonging to it are nonpathogenic. The second group is intermediate both in power of fermentation and in power of disease production. The third subgroup has the lowest fermentative power and has belonging to it a considerable number of highly pathogenic bacteria.

The Colon or Colon Aërogenes Subgroup of Bacteria

The most important species belonging to this subgroup are *Bacterium coli, Bacterium acidi-lactici, Bacterium communior, Bacterium neapolitanum, Bacterium coscoroba, Bacterium cloacæ* and *Bacterium aërogenes*.

The *Bacterium coli* and the *Bacterium aërogenes* are more important than the other species and are the only ones which will be discussed in detail. The differentiation

of the other species may be determined by reference to the key given below.[1]

Bacterium coli

This organism is taken as a type of the entire colon group. The other organisms belonging to the subgroup may be differentiated from it by means of physiological reaction. Inasmuch as most of them are intestinal forms, differentiation is usually not desired or necessary. In recent years differentiation of *Bacterium aërogenes* from *Bacterium coli* has been found to be of some importance in water analysis, inasmuch as the former seems to be much more widely distributed in nature and is not therefore as delicate an index of fecal contamination.

Synonym.—*Bacillus coli communis.* Colon bacillus.

Distribution.—*Bacterium coli* is normally present in the alimentary tract, particularly in the colon, of man and most animals. It is apparently not widely distributed in

[1] Key to the More Important Species of the Colon Subgroup of the Genus Bacterium

A. Ratio of $CO_2/H_2 = 1/1$, Voges-Proskauer test negative, methyl red (Clark and Lubs test) positive.
  1. No acid or gas from sucrose.
    a. Acid and gas from salicin.
      1. *Bacterium coli.*
    b. No acid or gas from salicin.
      2. *Bacterium acidi lactici.*
  2. Acid and gas from sucrose.
    a. Motile.
      3. *Bacterium communior.*
    b. Nonmotile.
      (1) Acid and gas in salicin.
        4. *Bacterium neapolitanum.*
      (2) No acid or gas in salicin.
        5. *Bacterium coscoroba.*

B. Ratio of $CO_2H/_2 = 2/1$, Voges-Proskauer test positive, methyl red test negative.
  1. Motile, liquefying gelatin, no gas or acid from starch or glycerol.
    6. *Bacterium cloacæ.*
  2. Nonmotile, not liquefying gelatin, gas and acid from starch and glycerol.
    7. *Bacterium aërogenes.*

nature except in places or localities fouled by the excretions of the body. It is very easily cultivated in the laboratory and it is probable that under certain conditions in nature it may multiply for a brief period of time outside the body. This, however, does not commonly occur, at least not in virgin soil or in unpolluted water. It is so abundant in feces that it has come to be looked upon generally in England and America as an indication of fecal contamination when found in water. It is not because *Bacterium coli* itself is pathogenic, but because it is an indicator of the presence of

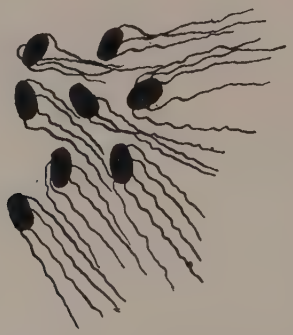

FIG. 66.—BACTERIUM COLI.

such fecal pollution that water containing it is generally condemned for domestic use.

Morphology.—The organism is a plump rod 0.4–0.7μ $\times$ 2–4μ. When growing rapidly it may be coccuslike. It rarely occurs in chains. Spores and capsules are not produced. It is actively motile, stains readily and is Gram-negative.

Cultural Characters.—The organism grows readily upon most culture media. Gelatin is not liquefied. Bouillon is clouded, sometimes with the formation of a pellicle. The growth upon potato is moist and spreading, the potato becoming darkened, sometimes almost black. Milk is coagulated with the formation of lactic acid and gas; the curd

is not digested. Upon agar plates the colonies are moist, opaque, becoming darker and more coarsely granulated. Gelatin is not liquefied.

Physiology.—*Bacterium coli* grows readily at blood heat and also at room temperature. Acid and gas are produced from dextrose and lactose. The typical *Bacterium coli* does not produce gas from sucrose but there are closely related species which show this reaction. It is readily destroyed by heat. The gas produced from carbohydrates is a mixture of equal volumes of carbon dioxide and hydrogen. In a suitable glucose peptone medium sufficient acid is produced so that methyl red is changed to a deep red color. Indol is usually produced in Dunham's solution. The Voges-Proskauer test is negative. The organism is *aërobic* and *facultative anaërobic.*

Pathogenesis.—Although *Bacterium coli* is usually regarded as nonpathogenic, there is reason to suppose that occasionally virulent varieties are developed which may produce disease in animals, particularly the so-called calf scours or calf diarrhea.

Recognition.—The physiological and cultural characters of this organism make it comparatively easy to recognize when present in the water and to isolate from sewage. The methods used will be discussed at greater length under the heading of Bacteriology of Water and Sewage.

Intermediate or Enteritidis Subgroup

Several species of bacteria belonging to this subgroup are of economic importance because of their association either as primary cause or secondary invaders with several diseases of man and animals. Among them are the *Bacterium paratyphi* and *Bacterium schottmülleri* causing paratyphoid fevers, the *Bacterium enteritidis* associated with food poisoning, *Bacterium morgani* which has been found associated with a typical dysentery in man. *Bacterium pul-*

lorum is the cause of the so-called white diarrhea of chicks. *Bacterium cholerœ-suis* has been found frequently associated with hog cholera but is evidently not the cause of the disease, in most cases at least. The following key will differentiate the species.[1]

Bacterium cholerÆ-suis

Synonyms.—*Bacillus suipestifer; Bacillus cholerœ-suis, Bacillus salmoni.*

This organism was first described in 1885 by Salmon and Smith as the probable cause of the disease named by them swine plague. A revision of terminology with reference to the swine diseases a little later caused them to describe this as the cause of the hog cholera. It was isolated from a large number of hogs affected with this disease. Much work was done with it in the next two decades in an attempt to produce vaccine and sera for use in treatment or prevention of the disease, hog cholera. In general these proved unsuccessful. It was not until De Schweinitz and Dorset in 1904 studied an outbreak of hog cholera in which they were unable to isolate this organism that the causal relationship

[1] Key to the Principal Species of the Intermediate Subgroup of the Genus Bacterium

A. Producing gas from mannitol.
  1. Producing gas from xylose.
    a. Lead acetate medium not blackened. Arabinose and dulcitol fermented but slowly if at all.
      1. *Bacterium cholerœ-suis.*
    b. Lead acetate medium blackened. Arabinose and dulcitol fermented promptly.
      (1) Inosite not fermented.
        2. *Bacterium enteritidis.*
      (2) Inosite fermented.
        3. *Bacterium schottmülleri.*
  2. Not producing gas from xylose.
    a. Nonmotile.
      4. *Bacterium pullorum*
    b. Motile.
      5. *Bacterium paratyphi.*
B. Not producing gas from mannitol.
      6. *Bacterium morgani.*

of this organism was brought into question. They proved that the disease could be produced by the injection of blood (from diseased animals) which had been filtered through fine grained porcelain filters. This procedure was known to remove the larger bacteria such as the "hog cholera bacillus" and demonstrated the disease to be due to one of the so-called filter-passers. Subsequent work showed quite conclusively that *Bacterium choleræ-suis* is not the cause of hog cholera generally, although it may be an important secondary invader and possibly in young pigs produces a disease somewhat resembling hog cholera. The organism

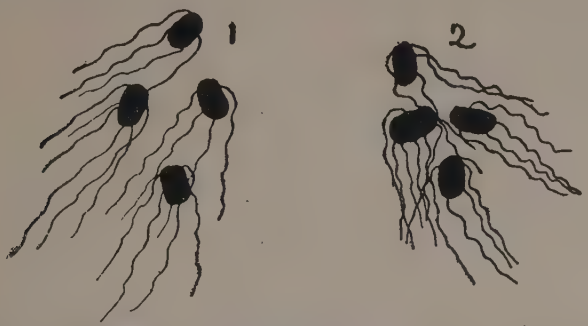

FIG. 67.—BACTERIA. 1. Bacterium cholerae suis. 2. Bacterium enteritidis.

can be differentiated from other members of the group only by its physiological and serological reactions.

BACTERIUM ENTERITIDIS

Synonym.—*Bacillus of Gärtner.*

This organism has been repeatedly isolated since its discovery in 1888 by Gärtner in outbreaks of food poisoning, particularly of meat poisoning in Saxony, and likewise from the uncooked flesh of a cow which had been responsible for the infection. It is possible that either this organism or forms closely related are responsible for many cases of calf diarrhea. Mohler and Buckley have also observed fatal

infection by this organism in cattle. It is responsible for many of the falsely termed ptomaïne poisonings in the United States.

The organism can be differentiated from other members of the group only by careful physiological and serological studies.

The *Bacillus enteritidis,* when grown in suitable culture media, produces an unusually soluble and potent endotoxin. This endotoxin differs from true toxin in being quite resistant to heat. It is therefore possible for food, particularly meat which has been fairly thoroughly cooked, to give rise to toxic symptoms when it is eaten. It is probable that this endotoxin is responsible for the symptoms which develop promptly when people are poisoned by eating infected food. The disease produced by infection by the living organisms in man is very similar to that caused by infection by paratyphoid bacilli, in fact, the organisms themselves are very closely related.

Meat and milk from animals which show severe diarrhea or gastrointestinal disturbances should not be used for human consumption, as infection in the human has been traced to such in several cases. It is quite probable that most of the cases of meat poisoning originate from the use of flesh of diseased animals, but it is possible that in some cases, the organism gains access to the meat after the animal has been slaughtered.

Bacterium paratyphi and Bacterium schottmülleri

Synonyms.—*Bacillus paratyphosus A* and *Bacillis paratyphosus B.*

These organisms produce in man the disease which has come to be known in recent years as paratyphoid fever. This disease closely resembles typhoid fever in most of its clinical aspects, although the mortality is somewhat lower. *Bacterium paratyphi* was originally discovered from the

fact that in certain cases, clinically typhoid, the blood serum of the patient did not acquire the property of agglutinating true typhoid bacteria, but that organisms could be isolated from the feces of the patients which would be agglutinated specifically by their blood serum. In recent years there have been recorded many instances of epidemics caused by the paratyphoid bacilli. The disease is transmitted in the same manner as is typhoid fever and methods of prevention are identical. These will be discussed at greater length under the heading of typhoid.

The bacteria causing paratyphoid fever of the two types can be differentiated from each other and from other members of this subgroup only by their physiological and serological characters.

Bacterium pullorum

This organism has been described as the specific cause of the disease white diarrhea in young chicks. It is similar in all but a few of its physiological and serological reactions to other members of this group. It is a nonmotile rod.

The disease is characterized in chicks by emaciation and white diarrhea. The organism may be isolated in pure culture from the internal organs, particularly from the liver. It has been found by Rettger that pullets which recover from this disease frequently develop into hens which are bacillus carriers, that is, the organisms remain in the ovaries and are present in the eggs laid. The chick, therefore, in such cases is infected before hatching. A few such chicks among those hatched by an incubator or present in a brooder will rapidly infect the other chicks. Infected hens may frequently be detected by means of the agglutination reaction, that is, their blood serum contains the agglutinin specific for the organism and the precentage of loss among chicks may be materially reduced by weeding out hens which react. The disease has been the occasion of very

considerable losses to the poultry industry in the eastern states.

Typhoid-Dysentery Subgroup

At least ten to fifteen species of bacteria are known which belong to this subgroup. Only two among them, however, are important from the standpoint of disease production, *Bacterium typhi* and *Bacterium dysenteriæ,* the latter with numerous varieties. Two of the species, *Bacterium gallinarum,* the cause of fowl typhoid, and *Bacterium abortus,* the cause of infectious abortion in cattle, are of importance in disease production in the lower animals.

Bacterium typhi

Synonyms.—*Bacillus typhosus, Bacillus typhi, Bacillus typhi-abdominalis, Eberth bacillus, Eberth Gaffky bacillus.*

This organism was discovered by Eberth in 1880 in the spleens of those who had died of typhoid fever, and has since been conclusively proved to be the cause of this disease. It is one of the most widely distributed of the organisms producing disease in man, the disease being more or less prevalent in most sections of the United States.

Morphology.—This organism is a short, plump rod, usually from .5 to .8μ in diameter and from 1 to 3μ in length, motile by means of numerous flagella. It is noncapsulated and does not produce spores. It is Gram-negative and stains readily with the ordinary aniline dyes.

Cultural Characters.—*Bacterium typhi* grows readily upon most of the cultural media, the growth being somewhat more delicate, usually, than that produced by *Bacterium coli.*

Physiology.—*Bacterium typhi* grows best at blood heat, but develops readily at room temperature. It is aërobic and in the presence of suitable sugars a facultative anaërobe. Acid, but no gas, is formed from dextrose, and neither acid

nor gas from lactose or sucrose. Milk is not coagulated and proteolytic enzymes are not produced.

The cultural and physiologic reactions make it possible to isolate the typhoid bacillus directly from the stools of infected individuals and, by a process somewhat more arduous, from infected water or sewage. Many special differential media have been developed to permit the ready isolation and identification of the organism. When pure cultures have been secured, the final test usually employed is agglutination by means of a specific anti-typhoid serum.

Disease Produced.—Typhoid fever is a disease characteristic of man and is not produced readily by either injection or by feeding of cultures to laboratory animals. The organism apparently develops first in the contents of the intestine, then invades the intestinal wall and the Peyer's patches. These latter frequently become ulcerated and occasionally perforation of the intestinal wall may result. The organisms are present in considerable numbers in the blood stream; in fact, the disease is frequently characterized as a bacteremia. The spleen is considerably swollen. Infection of the gall bladder is not infrequent.

The typhoid bacteria leave the body almost exclusively with the stools and occasionally with the urine. The bacteria do not multiply to any extent (if at all) in nature outside of the body; in fact, they die out with considerable rapidity after leaving the alimentary tract. Infection of another can occur only by swallowing typhoid germs. It is evident, therefore, that the prevention of typhoid fever depends upon preventing the ingestion of typhoid bacilli which have been voided comparatively recently by those having the disease or by so-called bacillus carriers. A bacillus carrier is a person who harbors pathogenic organisms such as typhoid bacilli without showing symptoms of disease. Usually those who have typhoid fever eliminate the organisms completely and do not show them in their

stools within a few weeks after the beginning of convalescence. Occasionally, however, the bacterium may persist for considerable periods of time. This is particularly true when there is an infection of the gall bladder. Such individuals have been known to retain the organisms for many years; they are, of course, much more dangerous in a community even than those who have the disease typhoid in clinical form, inasmuch as they have much greater opportunity to infect drinking water, food, milk, etc.

Transmission.—It is evident that typhoid fever must most commonly be contracted from drinking water, milk and other foods which have been contaminated. It is probable that not infrequently flies may carry the bacteria from the stools of typhoid patients to food.

Recognition of the Disease.—Typhoid fever is a disease which is not readily differentiated from other diseases in its earliest stages and with difficulty at any time in mild cases. As an assistance to the physician in recognizing the disease, the agglutination or Widal reaction has come into common use. A drop of blood or blood serum from the typhoid patient will usually agglutinate the typhoid bacillus in dilutions of at least 1 to 40, frequently in a dilution of one to many thousand. The disease may also be recognized by planting blood drawn from a vein in broth.

Prevention of Disease.—The prevention of typhoid fever is accomplished by preventing the access of fecal material to water and food, by the recognition and proper care of patients and carriers, and by the use of vaccines. The latter are necessary probably only where there is danger of the individual drinking water or eating food which has not been properly protected. The vaccine is prepared by growing typhoid bacilli on the surface of agar, washing them off with physiological salt solution, killing by heat, and standardizing to proper dosage. In some cases the bacteria are dried, ground and mixed with oil to make the so-called

lipovaccine. Vaccination has proved quite successful in preventing a high incidence of typhoid fever in large groups of individuals, such as soldiers exposed to infection.

Bacterium dysenteriæ

A considerable number of closely related species of bacteria are here grouped together. They produce diseases quite similar clinically, but the bacteria are specific and may be differentiated from each other by their fermentation and serologic reactions. The organisms all closely resemble the typhoid bacillus; they are, however, nonmotile. Methods of isolation are very similar to those used in typhoid.

In dysentery in man the intestine, particularly the colon, becomes inflamed and sometimes ulcerated. The stools are usually thin and watery. In most cases the bacteria (unlike typhoid) do not invade the general blood stream, that is, the disease does not develop into a bacteremia.

Transmission.—Dysentery is spread in quite the same manner as is typhoid and methods of prevention in general are identical.

Bacterium abortus

Synonyms.—Abortion bacillus of Bang, *Bacillus abortus, Bacillus abortivus.*

This organism has been repeatedly isolated as the probable cause of infectious abortion in the cow. The disease is prevalent in many places in Europe and in the United States in herds of dairy cattle. It should be noted that other organisms besides the one discussed here are found occasioning abortion in cattle and in other animals.

Morphology.—The organism is a small rod .3–.8μ×1–2μ. It is somewhat polymorphic in culture media, and involution forms are not infrequent. It is nonmotile, does not produce capsules or spores, and stains readily with aniline dyes. Frequently polar granules may be observed. This

has caused some investigators to place the organism in the genus *Pasteurella.*

Cultural Characters.—The isolation and cultivation of this organism are somewhat complicated by its peculiar relationship to oxygen. When in shake culture in serum agar, the organism grows in a layer several millimeters below the surface, that is, while not a strict anaërobe, it does not grow well (at least upon first cultivation) in the presence of an atmospheric pressure of oxygen; in other words it is micro-aërophilic. After cultivation for a time in the laboratory, it may be grown readily in the presence of oxygen in the ordinary laboratory media. It grows best with the addition of serum to the medium. The colonies develop at 37° as small, usually transparent dots. Growth is very slow at room temperature.

Pathogenesis.—Experiments have shown that this organism will cause abortion in pregnant cows, both by injection and by feeding. Injection into the rabbit or into guinea pigs is followed by specific changes in the liver and spleen and by the development of arthritis.

Immunity.—Cows that have aborted one or more times frequently become immunized against disease. Various methods of vaccination and serum treatment have been attempted but have not been attended with general success.

The organism is not infrequently present in milk from infected cows. The disease is probably frequently transmitted by ingestion, perhaps also by coition.

Abortin prepared in an analogous manner to tuberculin has been used to some extent for diagnosis. More frequently the agglutination test is used and has come to be the recognized test for diseased animals.

CHAPTER XXVI

HEMORRHAGIC SEPTICEMIAS AND PLAGUE—THE GENUS PASTEURELLA

The genus *Pasteurella* includes all of these bacteria which are aërobic, nonmotile, Gram-negative rods, not producing spores which show usually a decided tendency toward polar staining and possess a comparatively slight power of fermentation. The organisms belonging to this genus are closely related to certain forms of the genus *Bacterium* and in some cases it is quite difficult to differentiate between the two genera. The first organism described belonging to this group was that of chicken cholera, studied by Pasteur. Other organisms closely related were found later and Lignieres created the genus *Pasteurella* to include them all. The term *pasteurellosis* is a designation of the disease caused by any member of this group.

Bacteria of this group have been found associated with a great variety of diseases in animals and in man. The most important are the *hemorrhagic septicemia* of cattle and of sheep; the *septic pleuropneumonia* of calves; *fowl cholera; swine septicemia* and *swine plague,* and *bubonic plague* in man. The organisms causing these diseases in various animals are so closely related and so difficult if not impossible to differentiate in the laboratory, that some authors have regarded all as belonging to a single species and have designated as varieties merely those forms of causing diseases in the various animals.

In the present discussion the hemorrhagic septicemia of birds caused by *Pasteurella choleræ-gallinarum,* of cattle caused by *Pasteurella boviseptica,* of swine caused by *Pas-*

teurella suiseptica and of bubonic plague in man caused by *Pasteurella pestis* will be considered.

The organisms causing hemorrhagic septicemia in the lower animals and birds, as already mentioned, are so much alike that a description of one is satisfactory as a description for all except for pathogenic characteristics.

The Hemorrhagic Septicemias

It is probable that organisms belonging to this genus are in some cases nonpathogenic and that there has developed in literature some difficulty because of confusion of such nonpathogenic forms with the disease producers.

Pathology.—The pasteurellas are nonmotile rods, usually about $0.5 \times 1\mu$. The usual aniline dyes, particularly when the organisms are stained from body fluid, as blood, generally cause more intensive staining at the poles, the central portion of the cell scarcely staining if at all. Some forms when grown in culture media show frequently great variation in size and appearance, and the polar staining is much more difficult to demonstrate from such cultures than from tissues. The bacteria are Gram-negative and do not produce spores.

Cultural Characters.—These bacteria are readily cultivated in artificial media, producing upon gelatin and agar round, flat and grayish colonies, finally becoming grayish white and opaque. They do not grow well upon potato. In broth they may produce clouding with clearing by sedimentation; frequently a pellicle develops. There is no appreciable change in milk. The organisms show very slight fermentative powers. There has been an attempt to differentiate species on the basis of acid production from certain sugars, but this has not proved entirely successful.

Physiological Characters.—The organisms grow best at blood heat, but fairly well at room temperatures. They are aërobic, gas is never formed, but volatile acids may be.

Pathogenic Characteristics.—Practically all species of the genus *Pasteurella* quickly prove fatal when injected into the ear vein of a rabbit. The animal dies as a result of a septicemia; small hemorrhages usually may be noted in the membranes of the digestive tract. Practically all the species are pathogenic to sparrows. The various races show some differences in their ability to affect other species of animals. For example, fowls, in general, will succumb to various cultures of the organisms associated with swine plague, but not of the organisms causing hemorrhagic septicemia in cattle.

Pasteurella choleræ-gallinarum

Synonyms.—*Bacillus avisepticus, Bacillus choleræ-gallinarum, Bacillus choleræ,* and *Bacterium avicidum.*

This organism is the specific cause of the disease chicken cholera. This disease has a wide distribution over Europe and America. It was first studied by Pasteur in 1880. He succeeded in growing the causal organism in artificial media and made use of it in making studies on immunization. By growing this organism in artificial media he succeeded in attenuating certain strains so that they might be used as a vaccine. This work was of great historical importance, as it marked the first step in the study of experimental immunity, particularly the production of immunity by means of injections of pure cultures of microörganisms.

Apparently there are many different strains of the fowl cholera organism, showing marked differences in virulence. Some, when injected in considerable quantities into fowls, cause the development of a high degree of immunity; others bring about practically no immunity. The pigeon is very susceptible and is frequently used in a study of this organism in the laboratory.

Fowls which have died of chicken cholera usually show congestion of the heart and an accumulation of serum in

the pericardium. The liver is usually congested and shows small hemorrhages. The spleen is enlarged and softened and usually numerous petechial hemorrhages are to be found in the internal organs and the mucous and serous membranes.

Practical methods of immunization against the disease have not been developed, although there has been much experimental work done. The disease apparently is transmitted from bird to bird by ingestion of food or water fouled with excretions which contain the specific causal organism. The only practicable method of control apparently is careful sanitation.

Pasteurella boviseptica

Synonyms.—*Bacterium bovisepticum, Bacillus bovisepticus, Bacillus bipolare multicidum, Bacillus bovicida, Bacillus vitulisepticus.*

The disease hemorrhagic septicemia is one of the most widely distributed in the United States. Similar diseases have been described in European countries, both in domestic cattle and wild animals, particularly the deer. The disease in cattle is characterized by a fever due to the development of the causal bacteria throughout the blood stream and in the tissues. Upon post mortem, the animal generally shows large numbers of hemorrhagic spots or points in the internal organs and in some cases under the skin. These hemorrhages are particularly characteristic of the serous membranes. In some cases the hemorrhages in the subcutaneous tissues may be quite extensive and cause darkening of the skin.

It is possible to immunize animals by the injection first, of nonvirulent and later of virulent organisms, and the serum from such animals is known to have the power of producing passive immunity. It is evident, however, that there are many different strains of bacteria belonging to this species

and immunity against one is not necessarily potent against another. An attempt has been made to use a polyvalent serum, that is, one secured by injecting many strains of organisms into the animal producing the serum, or by mixing the serum secured from several animals immunized each against several strains. In some cases the serum has appeared to be effective in curing the disease in its early stages. Bacterins, that is, killed cultures of this organism have been quite extensively used in preventing the disease. The disease, however, is sporadic and appears and disappears with such suddenness that it is very difficult to evaluate the advantages of such treatment.

The disease is particularly apt to appear among young animals in first class condition.

Pasteurella suiseptica

Synonyms.—*Bacillus suisepticus, Bacterium suicidum, Löffler-Schütz bacillus* and *Bacillus suicida.*

This organism was usually described until recently as the specific cause of the disease swine plague. In the early history of the disease there was much confusion between swine plague and hog cholera. The discovery that hog cholera is caused by a filterable virus made necessary critical examination of the disease swine plague. Undoubtedly in many instances, this organism is a secondary invader in true hog cholera. However, there seems to be ample evidence that there is a specific disease which may be known as swine plague or swine septicemia caused by this organism and distinct from hog cholera.

Hemorrhagic Septicemias in Other Animals

Bacteria belonging to the hemorrhagic septicemia group have been found to cause diseases of this general type among many animals other than those already enumerated. Rabbit septicemia or rabbit plague is caused by *Pasteurella*

cuniculicida. Hemorrhagic septicemia has been described in the sheep and the horse, likewise an infectious pneumonia of goats, a pasteurellosis of buffalo, and pasteurelloses of many species of birds, including geese and ducks.

Pasteurella pestis

Synonyms.—*Bacterium pestis, Bacillus pestis bubonicæ.*

This organism is the specific cause of the disease plague in man and in rodents. It was discovered independently by Yersin and by Kitasato in 1894. The disease is one which is apparently endemic in certain parts of Asia, particularly in northern and western China, and has at various times spread over the entire civilized world. Bubonic plague has been reported as present from the ports of entry of practically every continent in recent years.

Pasteurella pestis resembles closely the other organisms belonging to this group. It grows rather readily upon culture media.

The disease plague in man is usually contracted from diseased rodents, particularly rats. It seems to be conclusively demonstrated that the disease is primarily one of rodents transmitted to man by means of the rat flea. If it attacks man, it may develop in one of the three forms. Bubonic plague results from cutaneous infection as a result of a bite from a flea or of scratching the dejecta of the flea into the skin. The organism enters the lymph channels and causes ulceration of the lymph nodes, that is, it forms so-called buboes. These may ultimately heal and the patient recover; more frequently, however, the organism finally invades the blood stream and the patient dies from the resultant septicemia. When the organism is more virulent it may enter the blood stream promptly after entrance into the body and produce a typical hemorrhagic septicemia. Hemorrhages develop under the skin and large areas become discolored. This is the so-called "black death"

which devastated many parts of Europe at the close of the Middle Ages. The disease may also be communicated to man by inhalation, developing as a very fatal type of pneumonia. Inasmuch as the organism is killed by drying, transmission in this manner usually takes place when patients and others come in contact in damp, ill-ventilated quarters.

It is possible successfully to immunize man against plague by injections of killed or attenuated bacteria or their products. It is also possible to immunize horses and use their sera in combating the disease. Inasmuch as there is little transmission to man except from the rat, it is necessary to conduct campaigns against these rodents wherever there is danger of an outbreak of a disease. In the United States, outbreaks have been controlled in this fashion in San Francisco, New Orleans and other ports. In some cases, as in San Francisco, it was found that the disease eventually attacked other rodents such as ground squirrels and wood rats. When once the rodents native to these regions become thoroughly infected, it becomes increasingly difficult, therefore, to stamp out the disease.

CHAPTER XXVII

THE SPORE-BEARING RODS—THE GENERA BACILLUS AND CLOSTRIDIUM. ANTHRAX, BLACKLEG, MALIGNANT EDEMA, TETANUS, BOTULISM, AND GASEOUS EDEMA.

The bacteria belonging to this group are all rod-shaped organisms capable of producing endospores. They differ considerably in size, cultural characteristics, shape and position of spores, motility and fermentative reactions. The group includes practically all of the spore-bearing bacteria.

The genus *Bacillus* includes those organisms which are aërobic or facultative anaërobic, the genus *Clostridium* those which are anaërobic and micro-aërophilic. Most of the bacteria, but not all, belonging to the group are Gram-positive, although there is considerable variation in the rapidity with which decolorization will take place.

One member of the genus *Bacillus, Bacillus anthracis* (the cause of the disease anthrax in cattle), and several members of the genus *Clostridium* will be considered. The most important of the species of *Clostridium* are *Clostridium tetani,* causing tetanus or lockjaw in man and animals, *Clostridium chauvœi,* causing blackleg in cattle, the organism causing botulism, *Clostridium botulinum,* and a group of organisms found associated with gaseous edema, the most important of these being *Clostridium welchii.*

Bacillus anthracis

Synonym.—*Bacterium anthracis.*

This organism is the specific cause of the disease known as anthrax or splenic fever in cattle. In man the disease is

sometimes termed malignant carbuncle, hide-handler's disease and woolsorter's disease. The anthrax appears most commonly in sheep, swine and cattle and somewhat more rarely in horses and in man. The bacillus of anthrax is of importance from a historical point of view as it was the first of the pathogenic bacteria to be accurately described. Pollender in 1855 stated that he had observed tiny rods in the blood of animals sick with the disease anthrax in 1849. In a publication in 1876, Koch completed the demonstration of the cause and relationship of this organism to the disease. This is of importance because it is the first record of the cultivation in the laboratory of an organism capable of causing disease and of the successful production of disease by the use of pure cultures. It is of interest further because Pasteur with it made the first successful application of the principle of vaccination by means of attenuated or weakened cultures of bacteria.

Distribution.—*Bacillus anthracis* is a strict parasite, that is, it does not grow widely distributed in nature. There are records of the occurrence of anthrax from the earliest times. Outbreaks have been noted throughout Europe and both Americas. In the United States there are still a few localities where the disease is enzoötic.

Morphology.—The bacillus of anthrax is a straight rod, occurring in chains, the individual cells usually showing truncate ends. The bacteria are usually 1–1.25 $\times$ 4.5–10μ. When examined in tissues or blood the chains usually are short, when grown in culture media the chains are long. The organism is nonmotile. Capsules are present in body tissues and in blood but are not readily demonstrated in culture media. Spores are produced when the organism grows in the presence of sufficient oxygen, but are not commonly found in the blood or tissues of animals with the disease. Each cell produces a single spore, oval or spherical in shape, which is located in the center of the cell and is of almost

the same diameter. The vegetative rods readily stain by the aniline dyes and are Gram-positive.

The organism culturally and morphologically very closely resembles the nonpathogenic and saprophytic species of the genus *Bacillus* as *Bacillus subtilis* and *Bacillus mycoides.*

Culture.—The *Bacillus anthracis* can readily be secured in pure culture from the blood or internal organs, particularly the spleen or liver, of infected animals. The colonies on gelatin or agar, when observed microscopically under the low power, are seen to consist of long chains of bacilli which resemble bunches of curled hair. This appearance is quite characteristic, but is not very unlike that observed in cultures of some of the saprophytic bacteria. In gelatin stabs filaments develop from the sides, giving rise to a characteristic spiking. The gelatin is slowly liquefied. Milk is first rendered slightly acid, then curdled and the casein finally digested. In broth a pellicle is developed which readily settles to the bottom and the medium is not usually clouded.

Physiology.—The organism is aërobic and facultative anaërobic. It grows best at blood heat, although growth may be secured at room temperatures. The maximum growth temperature is about 45° C. The rods are destroyed by heat, but the spores are more resistant, requiring a temperature approximately that of boiling water for some minutes. The latter are also very resistant to drying and have been kept alive for years dried in soil or upon threads. They are among the most resistant of the spores of the pathogenic bacteria. When pastures, therefore, have been infected with this organism, they may remain capable of transmitting the disease for a considerable period of years. Gelatin is digested.

Pathogenesis.—The organism is remarkably pathogenic for many of the laboratory animals. Mice are particularly susceptible, guinea pigs hardly less so, rabbits are

slightly more resistant. In most cases these animals will die from the result of infection in from 24 to 48 hours. Dogs and cats are somewhat less susceptible. Cold-blooded animals are refractory. Fowls are relatively immune.

Anthrax in cattle is usually marked by acute fever with not many external symptoms. Difficulty in respiration, bloody urine, and bloody discharges from the various body openings is common. The disease is usually fatal, the animal dying within a short time after the first visible symptom. Sheep are quite susceptible and the disease proves fatal very quickly. Horses having the disease in acute form also die within a few days. Occasionally in the horse, infection will occur through the skin and lead to the development of a so-called malignant carbuncle. Swine are somewhat more resistant and carbuncles and localized glandular infections are more common.

Anthrax in man is varied in character, dependent upon the method of infection. The so-called *woolsorter's disease* is a pulmonary infection with anthrax resulting from the breathing in of the spores by those who handle pelts from sheep which have died of this disease. This type of disease is always fatal. *Cutaneous,* or *skin anthrax* or *malignant carbuncle* is the more common infection in man. Such infection may result from contact with animals having the disease and has appeared repeatedly among those who handle hides, particularly hides which have not been adequately disinfected. During the great war there were many cases of anthrax in the army, both British and American, due to anthrax spores on shaving brush bristles. Apparently the horse hairs used for shaving brush manufacturers were secured from China and had not been adequately sterilized to insure the destruction of the anthrax spores which were present. The lesion at first resembles a boil, but the tissues round it soon begin to show infiltration, and not infrequently invasion of the blood and death from septicemia

follows. Radical intervention and the removal of the carbuncle is usually carried out.

In animals which have died of anthrax there is usually to be noted a considerable enlargement of the spleen and a decided softening of its pulp. Usually there are also hemorrhages and hemorrhagic exudates. Hemorrhages usually appear in the liver and lungs.

Immunity.—As previously noted immunization against anthrax was first developed by Pasteur. This investigator found that when the organism was grown in pure culture at temperature decidedly above the optimum, usually from 42° to 43° C. for varying lengths of time, the ability to produce disease gradually diminished. Cultures could be finally secured which when injected into rabbits would no longer kill them, but which were sufficiently virulent to kill guinea pigs and mice. Still longer cultivation at this high temperature will attenuate or weaken the organism still more, until guinea pigs are only occasionally killed by injection. Vaccination in sheep and cattle is most commonly performed by the injection of a highly attenuated culture; after the subsidence of all reaction, a culture somewhat less attenuated is injected as the second vaccine. As a result of the methods introduced by Pasteur, the disease anthrax has ceased to be a serious menace to the live stock industry of France and other European countries.

It is possible to secure an active antiserum by the hyperimmunization of animals by the injection of attenuated, then of more and more virulent cultures.

Transmission.—Anthrax is usually transmitted from animal to animal with feed, more rarely through lesions in the skin. Infection by inhalation and through the skin are most common in man. Animals which have died of anthrax should be completely destroyed by burning or by deep burying. This organism is peculiar in that it does not form spores within the body away from the air, but spores may

form in bacteria thrown off in bloody exudate or in the feces. In some cases, particularly in the southern United States, bloodsucking flies have been found to be of importance in transmitting the disease by direct inoculation.

Clostridium tetani

Synonyms.—*Plectridium tetani, Bacillus tetani.*

This organism is the cause of disease tetanus or lockjaw in man and many species of domestic animals. It was first observed by Nicolaier in 1885, who isolated it from animals that had died as the result of the injection of garden soil. The organism was cultivated in pure culture by Kitasato, who noted that it was an obligate anaërobe, that is, it will not grow in the presence of free oxygen.

This organism is not an obligate pathogen. It appears that it, in common with other members of the genus *Clostridium,* grows in the alimentary tract of animals, particularly of the horse, and only under exceptional conditions is able to produce disease. It has long been noted that the *Clostridium tetani* is found most frequently in soils which have been heavily manured. It is possible that it may maintain a saprophytic existence and even multiply to some extent in soil under favorable conditions.

Morphology.—*Clostridium tetani* is a slender rod .5μ $\times$ 2–5μ in length, with rounded ends. Usually it occurs in short chains; it is actively motile in young cultures by means of numerous peritrichic flagella. Spores are produced in abundance. The spore is terminal, spherical or approximately spherical and greater in diameter than rod which produces it. This gives a characteristic drum-stick appearance. There are but few other bacteria, and these closely related forms, which can be confused upon microscopic examination with this organism. It stains readily with the usual aniline dyes and is Gram-positive.

Cultural Characters.—The fact that *Clostridium tetani* is an obligate anaërobe makes its isolation in pure culture somewhat difficult. Colonies grown in gelatin are usually woolly. Bouillon is clouded and a sediment is formed. Blood serum is liquefied and milk more or less completely digested. Brain medium is blackened as a result of the development of hydrogen sulphide in the presence of iron.

Physiology.—The organism grows best at body temperature but readily likewise at room temperatures. It requires for the best development almost complete exclusion of oxygen. It will grow readily in mixed cultures with some organism which is an obligate aërobe. The spores are quite resistant and will withstand drying indefinitely. High temperatures maintained for considerable periods of time are requisite for certain destruction of the organism. Gas and acid are produced in small quantities from carbohydrates.

Pathogenesis.—The tetanus bacillus usually produces disease as a result of its inoculation into the tissue of a wound under conditions favoring the exclusion of air. The fact that it is not an obligate pathogen is well illustrated by the fact that when its washed spores are injected into the tissues, they usually do not produce tetanus, but are eventually destroyed by the body. It is usually only when this organism is associated with dirt and is to some extent protected that it multiplies and produces its characteristic toxin and symptoms of disease. It will also produce fatal results when injected in pure culture together with a little of the medium (containing toxin) in which it has been growing.

The *Clostridium tetani* when growing in a wound produces a powerful toxin. This travels apparently through two channels to the central nervous system. Some of the toxin passes into the blood stream and is absorbed from it by the central nervous system. Other portions travel along

the nerves. The symptoms of the disease are not apparent until the nerve cells have been injured as a result of the absorbed toxin. These injured cells stimulate the muscles (particularly the muscles about the area infected) to spasmodic contraction (tetanus). In man and some animals the nerve centers governing the muscles of the face are particularly apt to be affected and the contraction of the muscles causes the characteristic "locking" of the jaw.

It is apparent that an incubation period is necessary between the inoculation of organisms into the wound and the development of the symptoms. This is the time necessary for the formation of the toxin and the injury to the central nerve cells. In man this incubation period averages about 9 or 10 days, in horse it varies from 4 to 20 days or even more.

Immunity.—The characteristic toxin of the tetanus organism may readily be prepared in broth in which it is grown under anaërobic conditions. In commercial manufacture of antitoxin, this toxin is first titrated by determining the smallest amount which will certainly kill a 350 gram guinea pig in 2 to 4 days. This is called the unit of toxicity. At first small and then increasing doses of toxin are injected at suitable intervals into the horse; eventually the blood serum will be found to contain the specific antitoxin in considerable quantities. The animal is bled and the serum obtained in much the same fashion as has been previously discussed for diphtheria antitoxin.

The tetanus antitoxin is an excellent prophylactic, that is, when injected soon after the organism has been introduced into the body, it will neutralize the poison as it is formed and prevent the development of the symptoms of tetanus. When once, however, the symptoms have developed, the antitoxin is not always able to prevent a fatal result. In other words, antitoxin cannot undo the damage already done to the central nerve cells by the toxin taken up. It

can, however, neutralize other toxin as rapidly as it is developed.

Infection occurs almost invariably directly through an injury in the skin. Nail punctures in a horse are particularly apt to result in tetanus as they introduce the organism deep into the tissues and after the superficial healing with the exclusion of air which takes place, conditions become right for rapid multiplication. The statement that a rusty nail is particularly apt to produce tetanus is in the main true, inasmuch as it has much more opportunity to come in contact with dirt; furthermore, particles of rust will gain entrance to the wound and will not be brushed off by the skin as the nail enters.

Occasionally, particularly in the horse, there are instances of so-called cryptic infection in which it is not possible to demonstrate the wound through which the organism producing the disease has entered. It is possible that the organisms may circulate occasionally in the blood stream and be able to localize and grow only when the tissues have been injured in some fashion. This would explain, for example, the development of tetanus following a bruise or a fractured bone when the skin has not been broken.

Clostridium chauvæi

Synonyms.—*Bacillus chauvæi, Bacillus feseri, Bacillus anthracis symptomatici.*

This organism is the specific cause of the disease blackleg, symptomatic anthrax, quarter evil or quarter ill in cattle.

Morphology.—The *Clostridium chauvæi* is a relatively large organism, rod-shaped with rounded ends, rarely in pairs, usually single, 0.5 to 0.6 $\times$ 3–5μ. The organism, even in tissues, practically never occurs in chains. This is of some value in differentiation from the rather closely related *Clostridium œdematis.* The organism is motile by

means of peritrichous flagella. Under suitable conditions in cultural media spores are produced, sometimes centrally located but more frequently near the poles. The spores are elliptical in shape and cause the cell to swell somewhat. The organism is easily stained and usually Gram-positive.

Cultural Characters.—Culture is somewhat difficult because the organisms grow only under anaërobic conditions. Cultures grow best in a medium that is somewhat alkaline. Gas is produced from dextrose. Bouillon becomes clouded, and in the presence of carbohydrates, gas is produced.

Physiology.—The organism grows best at blood heat but readily also at room temperatures. It is strictly anaërobic; the spores are resistant to drying, having been found to live for years in this condition.

Pathogenesis.—Blackleg occurs most frequently in young cattle, but rarely in calves or in adult animals. The disease is characterized by swelling, softening, and gas production in the muscles and hemorrhagic edema of the parts which have been infected. It occurs most frequently in the shoulder or hind quarter. The presence of gas is usually demonstrable by the crackling sound which is produced when the thumb is drawn quickly across the skin of the infected parts. After the death of the animal the tissues may be generally invaded and the body become swollen due to the development of gas. When the tissues infected are sectioned they are found to be blackened and hemorrhagic. The disease is usually fatal.

Immunity.—According to some authors, the *Clostridium chauvœi* produces a true toxin, for which an antitoxin may be prepared. It has been found, however, that the antitoxin is of relatively little use in the immunization of animals. Much more commonly practiced is active immunization by means of vaccine. The standard method of preparation

adopted by the U. S. Department of Agriculture is to secure fresh muscle tissue in which the blackleg organism has been growing, grinding this in a mortar and finally squeezing out the fluid. This contains many of the organisms, together with spores. This is spread in a thin layer and dried to a brownish scale at blood heat. The vaccine is prepared by mixing one part of this material with two parts of water and placing in a hot air oven at a temperature of from 95° to 99° C. for six hours. This heating attenuates the organisms so that they may be injected without fatal results into susceptible cattle. The vaccine in some cases is suspended in water and is injected under the skin of animals to be immunized by means of a hypodermic syringe. In other cases it is made up into tablets which are inserted under the skin. In some cases, the vaccine is soaked on threads which are drawn into tissues by means of a needle. In general, vaccination against blackleg has proved satisfactory.

The complete life history of *Clostridium chauvœi* in nature is not well understood. It seems probable that it may under certain circumstances multiply in the alimentary tract of suitable animals. It is certain that the organism is present in some soils and not present in others. In some cases the pastures of one farm or one part of the farm may harbor the organisms, and such farms be subject to attacks of blackleg, and adjoining farms may be entirely free. In European countries it is well known that certain alpine pastures cannot be grazed by young cattle which have not been previously protected by vaccination against blackleg.

Infection is believed usually to occur through wounds and the disease is rarely if ever transmitted directly from one animal to another. Commonly it is difficult to determine the manner in which the organism has gained entrance, that is, cryptic infections are comparatively common. It is believed that in such cases the organism may

have gained entrance to the blood stream from the intestinal tract and have localized in some tissue which has been injured or bruised.

Clostridium welchii

This organism is taken as the type of a group of bacteria which in recent years have become very prominent because of their apparent relationship to gaseous and malignant edemas of various types in man and in animals. These bacteria are relatively common in many soils and when they gain access to wounds, produce a variety of lesions.

Clostridium welchii has been isolated repeatedly from men dying from gaseous edema.

Morphology.—The organism is a large rod about $1\mu \times 2$–6μ. It is frequently found in chains but may occur in pairs or groups. It is nonmotile. Spores may be produced under certain conditions in the laboratory upon culture media but are not produced in the presence of sugars. These spores are centrally located and the cells become considerably swollen and spindle-shaped. Capsules are produced in the body fluid and in some of the artificial media. The cells are usually Gram-positive.

Cultural Characters.—Colonies produced upon gelatin or agar plates are round, grayish and semiconfluent, resembling in many ways those of *Clostridium tetani*. Gelatin is usually slowly liquefied, bouillon clouded with the formation of abundant precipitate. Milk is quickly coagulated with the formation of gas and acid.

Physiology.—The organism grows best at blood heat. The thermal death point for organisms without spores, that is, for vegetative rods, is 50° c. for 10 minutes. Spores require boiling temperature for at least 15 minutes. An abundance of gas is produced from many of the carbohydrates including dextrose, lactose and sucrose but not from mannitol.

Pathogenesis.—This organism together with the *Clostridium œdematis, Clostridium Ghon-Sachs* and the *Clostridium sporogenes* and others may be present in soils, particularly cultivated and manured soils, whence they may gain access to wounds. In man these organisms are of special significance, because of their influence on war wounds, particularly "dirty wounds." Some species spread rapidly through the tissues, destroying them as they develop, causing hemorrhage, but not producing gas; others, as the *Clostridium welchii,* cause hemorrhages and produce an abundance of gas as well.

Immunity.—Certain of these species produce a specific toxin for which antitoxin may be prepared. Practicable methods of immunization have not yet been worked out, however. This is particularly difficult as bacteriological study including microscopic examination is necessary before one can know what particular species is causing edema in the particular individual and in consequence what serum should be used.

Diseases of this same general type have repeatedly been observed among animals, particularly sheep and swine.

Clostridium botulinum

Synonym.—*Bacillus botulinus.*

This organism is one of the frequent causes of meat and food poisoning in man. The disease is generally termed botulism from the Latin *botulus* meaning sausage. The organism was first isolated by Van Ermengen in 1896 from meat which he believed to be the cause of an outbreak of food poisoning. Poisoning caused by this organism should not be confused with that caused by *Bacterium enteritidis* or with the so-called ptomaine poisoning.

Distribution.—Outbreaks of poisoning of the type of botulism have been repeatedly reported from Europe, par-

ticularly in meat foods and sausages. In the United States practically all of the cases reported have been from the eating of imperfectly preserved canned foods. A considerable variety of these have been found to be at fault. Cases of poisoning from canned asparagus, canned beans and ripe olives have been reported. Most of the cases have been from the Pacific coast.

Morphology.—The *Clostridium botulinum* is a relatively large bacillus, usually single, occasionally in pairs or chains. It is .9–1.2$\mu \times$ 4–6μ. It is actively motile by means of from 4 to 8 peritichous flagella. Oval or elliptical spores are produced, one near the end of each cell. The organism stains readily and is Gram-positive.

Culture.—Care must be taken to exclude oxygen in the growth of *Clostridium botulinum*. It grows fairly readily in ordinary cultural media.

Physiology.—The organism is an obligate anaërobe. Some strains grow best at room temperatures, others at body temperature. Apparently there are several distinct varieties of this organism which show minor differences in physiological reactions. Gas is generally produced from dextrose but not from sucrose or lactose.

Pathogenesis.—When growing under favorable conditions, the *Clostridium botulinum* produces a highly potent toxin. Usually this is accompanied by the formation of malodorous substances, particularly butyric acid. Usually food in which this organism has been growing can be detected by having an "off" odor. Such foods must be carefully avoided as even a small portion of such food, sometimes not more than a mere taste, has proved sufficient to be fatal.

The toxin produced by growing the organism in culture media is quickly fatal to laboratory animals when fed or injected. It is also fatal frequently to man. In man the

symptoms include lack of coördination of the eyes (that is, double vision) and difficulty in control of the muscles governing swallowing.

Until recently it has been believed that the organism itself (free from toxin) when introduced into the animal body is nonpathogenic, but more recent work seems to show that the organism when injected or fed to certain animals is capable of producing serious or fatal results.

Limber neck of chickens may be caused by feeding food containing the organism or its toxin. The birds are partially paralyzed, resting the tip of the bill upon the ground and showing lack of ability to hold the head erect.

According to Graham and his coworkers, this organism is one of the common causes of so-called forage poisoning in cattle and in horses. It has been found that this organism could be isolated from silage and fodder, producing the disease called "blind-staggers" in the horse, and that the disease could be produced by feeding horses upon fodder inoculated with pure cultures of the organism.

Inasmuch as this organism produces a toxin, it is possible that the corresponding antitoxin could be used in treating the disease. In animals it has been used effectively in several instances and very possibly it may be of value in man. Recent work, however, indicates that there are at least two distinct strains of *Clostridium botulinum* whose toxins differ somewhat, and it is necessary therefore in successful treatment to have an antitoxin specific for the organisms which produce the infection.

Inasmuch as this organism has been found to infect man most frequently from canned foods, it is of interest to note that the disease has resulted both from the eating of commercially canned foods and home canned products. It is important that canned foods be adequately sterilized and care should be used not to consume foods which are "off"

in odor. Boiling will destroy the potency of the toxin and long continued boiling will usually destroy the organism and its spores. Recently cooked foods, therefore, are probably never dangerous.

CHAPTER XXVIII

THE GROUP OF RAY BACTERIA, LUMPY JAW AND RELATED INFECTIONS—THE GENUS ACTINOMYCES

Organisms belonging to the genus *Actinomyces* are very abundant in soil. Many different species have been described. A few only are known which under certain conditions are capable of producing disease in animals and occasionally in man. It is not at all improbable that these are really saprophytic forms which are able to adjust themselves to parasitic conditions.

The organisms belonging to this group are intermediate in most of their characteristics between the bacteria and the molds, and by many investigators they are included with the latter. They resemble the molds in producing chains of spores in many cases and also in producing a branched mycelium. In many forms these hyphæ may break up into short lengths and function in some cases at least, as spores. Only one species, the form producing lumpy jaw in cattle (*Actinomyces bovis*) is to be discussed here, although several other species have been described from somewhat similar lesions both in animals and in man.

Actinomyces bovis

Synonyms.—*Streptothrix bovis, Discomyces bovis.*

This organism and perhaps some closely related species are the specific causes of the disease lumpy jaw and wooden tongue or actinomycosis in cattle and probably in some cases in other animals. The disease lumpy jaw is known from many areas in Europe and North and South America.

Morphology.—When the purulent material from a case of lumpy jaw in cattle is examined there will be found many minute yellowish granules sometimes large enough to be observed by the unaided eye. Microscopic examination of these tiny grains show them to be made up of compact masses of the organisms arranged more or less radially. When the edge of the granule is examined carefully it will be found to be made up of club-shaped enlargements. The filaments in the middle of the granule are usually half a micron in diameter.

The organism stains readily with the common aniline dyes and is Gram-positive. When growing upon artificial media it consists of interlacing branched threads, forming a relatively compact mass.

Culture.—In broth the organism forms distinct spherical mulberrylike masses, generally near the bottom of the tube. Agar cultures, particularly those containing glycerin, show the development of colonies, which resemble tiny drops of amber, which may enlarge and may remain discrete or colorless, or form a distinctly wrinkled membrane, sometimes rather dusty in appearance.

Pathogenesis.—The organism does not appear to be pathogenic for most of the laboratory animals. In most cases the lumpy jaw or wooden tongue of cattle is primarily due to the presence of a splinter or grass awn which has worked its way into the tongue or the gums around the roots of the teeth. These evidently carry with them the spores of the *Actinomyces* which is then aided in invading the tissues. The swelling or tumorlike mass develops at the side of the infection. This may soften and ultimately discharge a yellowish pus. When the tongue is the primary seat of infection, it becomes enlarged, indurated or hardened and may protrude from the mouth.

The disease apparently is quite noncontagious—it is not at all readily transmitted from one animal to another.

Methods of immunization against the disease are not known or have not been developed.

Related Organisms

Microörganisms closely related to *Actinomyces bovis* have been found to cause one type of bovine farcy, actinomycosis in goats, in the dog, and Madura foot in man.

CHAPTER XXIX

THE ACID FAST GROUP—THE GENUS MYCOBACTERIUM—TUBERCULOSIS, PARATUBERCULAR DYSENTERY AND LEPROSY.

THE organisms belonging to this group are all slender, nonsporing rods, which do not generally produce capsules, are Gram-positive, and nonmotile. They also have in common the character of acid fastness, that is, they are relatively difficult to stain with the aniline dyes, but when once stained they retain the stain with avidity, not losing it even in the presence of relatively strong solutions of mineral acids. All of the species belonging to this group, furthermore, show a decided tendency under certain cultural conditions to produce elongated, more or less branched threads. In some respects they are intermediate between forms like *Actinomyces* and the other bacteria.

Organisms belonging to this group are widely distributed in nature, in soil, in manure, etc. Three species are of importance because of their ability to produce disease, that is, they are primarily pathogenic and parasitic. These are the *Mycobacterium tuberculosis*, the cause of the disease tuberculosis, *Mycobacterium paratuberculosis*, the cause of paratubercular dysentery in cattle, and *Mycobacterium lepræ*, the cause of leprosy in man.

MYCOBACTERIUM TUBERCULOSIS

Synonyms.—*Bacillus tuberculosis, Bacterium tuberculosis.*

This organism, together with its several varieties, is the specific cause of tuberculosis in man, birds and various

animals. Inasmuch as tuberculosis is a disease which may attack many parts of the body, the clinical symptoms may vary in different cases and the appearances on *post mortem* examination may differ widely. In consequence until modern times tuberculosis of different types were regarded as different diseases. Tuberculosis of the skin, tuberculosis of the lungs, tuberculosis of the peritoneum and tuberculosis of the bones and joints were regarded as distinct diseases. It was only after the discovery of the specific organism in 1884 by Robert Koch that the essential identity of the various types of the disease was established. He succeeded in showing the characteristic organism always to be present in nodules of the disease, and succeeded further in growing the organism in pure culture in the laboratory and reproducing the disease upon experimental inoculation. The organisms isolated from man, cattle and birds appeared so nearly alike that at first they were regarded as identical, that is, it was assumed that tuberculosis in all animals was caused by the same germ. In 1896, Theobald Smith observed that there were certain differences to be noted in the culture, morphology and physiology of the bacteria isolated from cases of human and bovine tuberculosis. This was further elaborated by Koch who in 1901 concluded that the organism causing tuberculosis in man was quite distinct from that of tuberculosis in cattle. At the present time at least four distinct varieties of *Mycobacterium tuberculosis* have been recognized. The variety most frequently causing tuberculosis in man is variety *hominis,* the type producing disease in cattle, variety *bovis,* the type commonly producing disease in birds, particularly domestic fowls, variety *avium,* and the type producing disease in cold-blooded animals, such as fishes and turtles, variety *piscium.* It seems to be well demonstrated that it is difficult if not impossible to transform one variety into another. The preceding statement should not be misinterpreted; it

is not impossible to produce disease in humans, for example, by means of bovine bacilli. The facts will be considered below.

Tuberculosis is usually the cause of more deaths in man in the United States annually than any other disease. The disease in cattle is widely distributed, particularly in dairy animals both in Europe and America. Swine, particularly those which run with the cattle, are often infected. The disease occurs occasionally in the horse and in the sheep. Tuberculosis of the domestic fowl is common in certain districts in the United States.

Morphology. — The *Mycobacterium tuberculosis* is a slender rod, frequently somewhat bent and with rounded ends. Not infrequently the protoplasm stains somewhat irregularly, giving a beaded appearance to the cell. Spores and capsules are not produced. It is Gram-positive and is nonmotile. The cells vary from .2 to .5μ in diameter and 1.5 to 3.5μ in length. When grown upon certain culture media elongated and branched forms are not at all uncommon. The organism is best stained with one of the more powerful analine dyes, such as carbol fuchsin. Apparently the acid fastness of this organism is due to the presence of a waxlike material in the cell walls.

There is some difference of opinion as to whether there are noteworthy differences in the morphology of the varieties of the tubercle bacillus. It has been claimed that organisms from bovine cases are somewhat shorter, straighter and thicker than those from human cases and are somewhat less apt to show the granular staining characteristic. The varieties, however, are much more satisfactorily differentiated by the use of cultural, physiological and pathological tests.

Cultural Characters.—The tubercle bacillus, at least when first isolated from the body, requires special media for its growth. Pure cultures may be secured by rubbing

nodules secured under aseptic precautions from infected animals over the surfaces of solidified blood serum or egg medium. It is sometimes necessary to keep at blood heat for one to two weeks before visible growth develops. When the organism is not in pure culture as is the case in milk, feces, etc., isolation is frequently attended with difficulty. It may be accomplished by inoculating the material from which the culture is to be secured into a suitable animal such as a guinea pig, allowing the disease tuberculosis to develop, and securing the organism in pure culture from the lesions. It may also easily be secured by dissolving the organic material and other bacteria in antiformin, washing and inoculating a culture tube.

After cultivation in the laboratory the organism will grow somewhat more readily upon culture media, particularly those which contain blood serum, egg or similar proteins or to which glycerol has been added.

The colonies upon blood serum or upon glycerin agar appear first as tiny grains barely visible to the naked eye. In cultures where they grow readily, the colonies become confluent and cover the surface of the medium with a dry, wrinkled growth. In old cultures this may become creamy or brown in color. In glycerin broth there is usually formed a more or less heavy wrinkled scum which readily sinks to the bottom when shaken.

Organisms isolated from human sources usually adapt themselves much more quickly and readily to culture media than those from bovine and always grow much more luxuriantly. In typical cases it is not difficult to tell which strain an organism belongs to by the differences in the growth appearance. The bovine organism usually forms isolated colonies while those from human cultures form a continuous wrinkled growth over the surface of the medium.

Physiological Characters.—The organisms from human and bovine sources grow best at blood heat and their growth

temperature range is relatively narrow. It is difficult to get growth below 30° C. or above 40° C. The bacteria are readily killed by heat, the thermal point being given as 60° C. for 20 minutes. Desiccation destroys the organisms slowly, although they have been known to remain alive in dried sputum for several months. Certain physiological characters have been noted as of assistance in differentiating the human and bovine tubercle bacillus. It was noted by Theobald Smith that when the human bacillus is grown in glycerin broth somewhat acid to phenolphthalein, it brings about a permanent acid reaction; while with the bacillus of bovine tuberculosis the acidity finally diminishes and the reaction eventually becomes alkaline.

Pathogenesis.—Tuberculosis is typically a chronic disease. It is characterized, no matter what part of the body is affected, by the development of nodules having eventually the same structure. When the tubercle bacillus lodges in a tissue it causes a multiplication of the connective tissue cells. These become surrounded by a more or less delicate layer of epitheloid cells while at the center there are so-called *giant cells* containing numerous nuclei. The whole mass is termed a tubercle or a *miliary tubercle*. Eventually autolysis of some of the cells in the interior of the nodule takes place and it undergoes what is termed caseation, that is, the interior becomes cheeselike in consistency. This is particularly apt to happen when nodules are found in large masses. In many cases the tissues surrounding the nodules develop a firm fibrous capsule, and this may eventually become infiltrated with lime, that is, *calcified*. Such granules will persist indefinitely in tissues which have been tubercular even after the disease has been entirely cured. The organisms are particularly apt to invade lymph nodes and glands through the lymph channels.

The different varieties of the tubercle bacillus show marked variations in their pathogenesis for animals. In

general the bovine bacillus is the most pathogenic, the human next and the avian least except for birds. Inoculation in guinea pigs with bovine bacillus usually leads to death in less than 30 days, while those inoculated with human bacillus will live for several months. Injection of the rabbit with bovine bacillus usually kills the animal within three weeks, while similar injection of the human bacillus usually does not prove fatal for several months and in some cases the animals may recover. Calves inoculated with bovine bacilli succumb rather rapidly to the disease, while similar inoculation with human bacilli produces only local lesions which eventually heal.

In man tuberculosis is most common as a disease of the lungs (so-called *consumption*). Next in frequency come tuberculosis of the lymph glands of the neck (scrofula), of the bones and joints (bone and articular tuberculosis), of the covering of the brain (tubercular meningitis), abdominal tuberculosis and occasionally tuberculosis of the skin (lupus). In cattle the disease generally attacks the mesentery causing the development of the so-called pearly disease characterized by the nodules on the membrane lining the peritoneal cavity. Tuberculosis of the lungs is also common. Frequently in the cattle the lymph nodes become enlarged. Tuberculosis of the udder is sometimes noted in tubercular cows. Swine generally show infections of the lymph glands in the neck, frequently also abdominal and pulmonary lesions. In the domestic fowl the tuberculosis is in general abdominal, the lesions and nodules showing particularly in the liver and spleen and somewhat more rarely in the lungs.

Immunity.—Practicable methods of immunizing against tuberculosis have not been developed although many attempts have been made and there has been an immense amount of systematic work done in this field. It is possible to immunize cattle against bovine tuberculosis by injection

of nonvirulent strains of the human type. The immunity, however, does not last for more than a year.

Diagnosis of the Disease.—Tuberculosis is most commonly recognized by taking advantage of the fact that animals affected with the disease show marked hypersusceptibility or anaphylaxis, that is, when an extract of the tubercle bacillus is injected into an animal having the disease a marked reaction generally results.

Such an extract of tubercle bacilli used for diagnosing the disease is known as *tuberculin.* The standard tuberculin most commonly used in the testing of cattle, the so-called *Koch's old tuberculin,* is prepared by inoculating a flat-bottomed flask containing 5 per cent glycerin broth to a depth of 2 to 3 centimeters with a culture of *Mycobacterium tuberculosis.* Careful inoculation of the surface from a pure culture and incubation at blood heat results in the growth of a film or pellicle. This is well developed in a few weeks. The broth is then evaporated in a porcelain dish on a water bath until the glycerin concentration becomes about 60 per cent, when it is filtered through paper or a porcelain filter. It is evident that the extract contains the growth products of the tubercle bacillus together with glycerin, salt and various substances present originally in the broth. Purified tuberculins and tuberculins made from the portions only of the tubercle bacillus have been prepared in a great variety of ways.

Diagnosis of tuberculosis in cattle is most frequently effected by the subcutaneous injection of tuberculin. The dose given is about one-fourth cubic centimeter of the Koch's old tuberculin. Before the injection of the animal the normal temperature should be determined. After six or eight hours the temperature is again taken and thereafter every two hours during the test. Animals in heat or advanced in pregnancy or suffering from other diseases should not be tested. In general a positive test will be

recognized by a rise of at least one and one-half degrees above the previous maximum recorded temperature. Usually the temperature begins to rise within 8 hours after injection and reaches its maximum in 18 hours, then gradually subsides.

In addition to the generalized tuberculin reaction local reactions are frequently used in disease diagnosis. Most common of these is the so-called *intradermal test* used in cattle. In this method a small amount of tuberculin is injected into the skin (not under the skin); most commonly this injection is made into the skin showing no hair at one side of the tail. The reaction consists of a circumscribed swelling about the size of a hulled walnut within twenty-four hours. A somewhat similar method may be used in swine, the injection being made into the ear lobe. An ophthalmic or eye reaction is occasionally used. In this test a tuberculin which has been prepared free from glycerin and other irritant substances must be utilized. It is dropped into the corner of the eye. The eye within twenty-four hours will become inflamed and a marked conjunctivitis will develop. This will disappear within three days.

The tuberculin test has been proved to be a remarkably reliable method of diagnosing tuberculosis in cattle. It has been used to some extent in diagnosing the disease in other animals as well. The tests used in general resemble those used in cattle. In man, particularly in children, it has been noted that rubbing tuberculin or tuberculin ointment into the skin vigorously will result in the development of a marked reddening at the site of the inunction in tuberculous individuals.

While the tuberculin reaction appears to be a reliable method of recognizing tuberculosis in cattle when carefully carried out, it should be noted that an injection of tuberculin may interfere with the thermal reaction of the animal

to tuberculin for a few days thereafter, or in some cases for a longer period. This fact has been made use of by dishonest cattlemen in securing certification of freedom from tuberculosis in animals. The veterinarian who makes the test, however, may overcome the results of such previous injection by the use of larger doses of tuberculin or by the use of the intradermal test.

It should be noted furthermore, that in some cases, animals that are in the last stages of the disease or are considerably emaciated may fail to show the reaction. The presence of large numbers of organisms constantly in the body has made it impossible for the animal to react normally to the injection of the tuberculin. Naturally the fact that animals showing very marked symptoms of the disease may occasionally fail to show the reaction while animals having only slight lesions, perhaps only an obscure nodule in a lymph node, may show marked reaction, has led to considerable skepticism on the part of practical farmers with reference to the test. When properly understood, however, it may be regarded as reliable.

Transmission.—Tuberculosis in man is transmitted most commonly by inhalation of infectious droplets thrown off in coughing, through contaminated drinking vessels and in some cases undoubtedly by the eating of food containing the tubercle bacillus. The bacteria leave the body with the sputum or feces and occasionally with the urine.

Tuberculosis exists in animals and man either as a *closed* or an *open* type. In closed tuberculosis the bacteria are present in organs or tissues that have no direct communication with the surface of the body. The presence of tubercle bacilli, for example, in the lymph nodes, would not necessarily lead to their being discharged by the body. In open tuberculosis the bacteria are growing in tissues which communicate readily with the surface of the body, in the lungs, in the liver or in the walls of the alimentary tract. In

SUMMARY OF TYPES OF TUBERCLE BACILLUS FOUND IN 1511 CASES

Diagnosis	Adults, sixteen years and over		Children, five to sixteen years		Children under five years	
	Human	Bovine	Human	Bovine	Human	Bovine
Pulmonary tuberculosis	778	3	14	..	35	1
Axillary or inguinal tubercular adenitis	3	..	4	..	2	..
Cervical tubercular adenitis	36	1	36	22	15	24
Abdominal tuberculosis	16	4	8	9	10	14
Generalized tuberculosis of alimentary origin	6	1	3	4	17	15
Generalized tuberculosis	29	..	5	1	74	7
Generalized tuberculosis and meningitis of alimentary origin	...	..	1	..	5	10
Generalized tuberculosis and meningitis	5	..	10	..	76	1
Meningitis	1	..	3	..	28	4
Tuberculosis of bones and joints	32	1	41	3	27	..
Other types	34	5	6	7	3	..
Totals	940	15	131	46	292	76

open tuberculosis the organisms are being thrown off more or less constantly by the body. In the closed type, the bacteria are not leaving the body and the disease is in this condition, noncontagious.

Cattle are probably most commonly infected by ingestion, perhaps occasionally by inhalation. Hogs generally show tuberculosis when they are following tubercular cattle and infection is undoubtedly by ingestion. The same apparently is true of tuberculosis in fowls.

Transmission of Bovine Tuberculosis to Man.—It has been noted that the bovine and human tubercle bacilli are relatively distinct varieties, and that the human tubercle bacilli do not readily infect cattle. The conclusion was reached by some investigators, therefore, that bovine tubercle bacilli do not affect man. In recent years, however, it has been shown that in certain types of cases, and particularly in children, bovine tubercle bacilli produce a considerable proportion of the cases. Evidently tuberculosis can be transmitted to man, therefore, by milk from tuberculous cows. The chart given on page 380 adapted from Park and Krumweide summarizes the types of tubercle bacillus producing disease in man in 1511 cases. An examination of this table would seem to prove quite conclusively that the tuberculosis of bovine origin is frequent enough in children under sixteen years to justify all reasonable precaution against the ingestion of infected meat and milk. On the other hand, the bovine tubercle bacillus is very rare as a cause of disease in adults.

Mycobacterium paratuberculosis

Synonyms.—*Bacillus paratuberculosis* and Bacillus of Johne's disease.

This organism is the cause of a peculiar chronic dysentery in cattle. The early investigators concluded the organism

to be identical with the tubercle bacillus which causes disease in fowls. It seems to be well established, however, that the organism is distinct. The disease has been noted in various parts of Europe as well as in the United States.

Morphology.—The organism closely resembles the *Mycobacterium tuberculosis*. In fact the two are indistinguishable under the microscope.

Cultural Characters.—The organism grows with difficulty and only upon special media.

Pathogenesis.—The disease in cattle is characterized by progressive emaciation and by a persistent chronic diarrhea, usually proving fatal. *Post mortem* examination shows the lesions to be confined largely to the intestines. In these the mucous membranes show thickening and wrinkling. Practical methods of immunization have not been developed. It is probable that the disease is transmitted by ingestion.

Mycobacterium lepræ

Synonyms.—*Bacterium lepræ* and *Bacillus lepræ*.

This organism is the specific cause of leprosy in man, a disease common in Asia, in certain sections of northern Europe and some of the Pacific Islands. Occasionally it has been found in the United States. A very closely related disease sometimes occurs in rats. The organism in many respects resembles the tubercle bacillus. The organisms may multiply in the nerves, causing loss of sensation or the so-called anesthetic leprosy, or they may multiply in the subcutaneous tissues, causing the formation of tubercles or nodules closely resembling those of tuberculosis. The disease is a chronic one and the individual may survive for many years. The disease apparently is nontransmissible to any of the lower animals.

Apparently leprosy is not highly contagious. The exact method of transmission is not known, it is probably accomplished as a result of direct contact. The organism does not grow readily upon artificial media.

The disease is successfully treated in many cases with Chalmoogra oil.

CHAPTER XXX

DIPHTHERIA AND GLANDERS GROUP—THE GENERA CORYNEBACTERIUM AND PFEIFFERELLA

Corynebacterium

The bacteria belonging to the genus *Corynebacterium* are rods, Gram-positive, nonmotile, aërobic and facultative, not producing spores and characterized by the possession of metachromatic granules. They may also show marked tendency to the development of threads and more or less irregular branching and club forms. The most important organism belonging to the group is the *Corynebacterium diphtheriæ,* the cause of the disease diphtheria. Another organism, the *Corynebacterium pseudotuberculosis,* has been found to produce a considerable variety of infections in domestic animals, the organism in sheep giving rise to the formation of enlarged lymph glands and producing somewhat similar diseases in cattle, horses, rabbits and other animals. In addition there have been described a considerable number of organisms belonging to this group, associated with other diseases in man but not definitely proved to have a causal relationship to them. Bacteria belonging to this group furthermore have been isolated repeatedly from the throat and mouths of healthy individuals. The only species which will be discussed is the organism causing diphtheria.

Corynebacterium diphtheriæ

Synonyms.—*Bacillus diphtheriæ, Bacterium diphtheriæ, Mycobacterium diphtheriæ.*

This organism is the specific cause of the disease diph-

theria in man. The term diphtheria was originally used by physicians to indicate a characteristic inflammation of the mucous membrane together with formation of a false membrane in the throat or nose. This particular type of infection was said to be diphtheritic. Inasmuch as the organism under discussion is the most common cause of conditions of this type in the throat, the term diphtheria is now generally recognized as applying only to lesions produced by this particular organism.

Morphology.—Young cultures of *Corynebacterium diphtheriæ* show rods predominantly 0.4–1.4μ in diameter and 1.5–3.5μ or more in length. They show marked variation in size and staining characteristics, depending upon the type of medium upon which they are growing and upon the age of the culture. Not infrequently they may stain uniformly, but more commonly there are deeply-stained bands or granules within the cells. Frequently the cells are slightly curved or pointed and club-shaped. Occasionally branched cells have been noted.

Cultural Characters.—The organism may be readily secured in culture by rubbing a swab first over the inflamed throat surfaces or upon the false membrane of the diphtheria patient and then over the surface of coagulated blood serum. When grown at blood heat upon this medium, development is very rapid; usually colonies may be observed in 12 to 18 hours as small pin-pointlike translucent dots, which later become opaque and grayish. It grows best on media containing blood serum or glycerin. It gradually accustoms itself to growth upon artificial media and develops more luxuriantly after many transfers. When grown in broth a delicate scum forms. It will be noted that this pellicle formation is essential in the manufacture of diphtheria toxin already discussed.

Physiological Characters.—The organism is aërobic and facultative anaërobic. The fact that it does not produce

spores makes it relatively nonresistant to drying at a high temperature. Gelatin is not liquefied. It is readily destroyed at the temperature of pasteurization.

Pathogenesis.—The disease diphtheria is one of the best examples of a true toxemia, that is, a disease in which a microörganism produces a powerful poison without itself invading the tissues to any great degree. It localizes usually in the throat, less commonly in the nose and still less frequently on other mucous surfaces and in wounds. Diphtheria as it attacks the throat produces a poison which causes the death of the epithelial cells of the mucous membrane. These are thrown off, leaving a raw surface, plasma is exuded, coagulation of the fibrinogen takes place and there is gradually built up a relatively thick membrane. In most cases the severe reaction in diphtheria is due to the absorption of the toxin. In some cases asphyxiation due to development of the false membrane may occur. In this membrane conditions are favorable for the growth of the microörganism and unless the body produces antitoxin rapidly or antitoxin is introduced artificially, the case will probably prove fatal.

A discussion has already been given of the method of manufacture of the diphtheria toxin and the antitoxin under the general heading of "Immunity." It is well known that persons that have had diphtheria once do not frequently contract the disease a second time; in other words, such individuals have an acquired immunity. An immunity conferred by the injection of the diphtheria antitoxin does not last for more than a few months at the most.

The disease diphtheria is usually diagnosed by means of cultures taken from the throat of the patient. Usually the medium employed is Löffler's blood serum. Mounts made from such slants after incubation for 8 to 18 hours, stained with methylene blue show the characteristic metachromatic granules. Occasionally it is possible to grow

the organism in practically pure cultures in smears taken directly from the throat.

Transmission.—Diphtheria is most commonly transmitted by the use of common drinking vessels or by passing objects infected in the mouth of one individual to that of another. It is possible that occasionally the disease may be transmitted by the inhalation of infectious droplets.

Disease carriers (or bacillus carriers so-called) are particularly important in this disease. In any outbreak or epidemic of diphtheria, a considerable proportion of children and other people who come in contact with diseased individuals may show no symptoms of the disease and yet harbor the organism in the nose or throat. Such individuals can transmit the disease to those who are susceptible quite as readily as those showing serious lesions. Such a diphtheria carrier in a community may readily constitute the starting point of a serious epidemic.

Genus Pfeifferella

The organisms belonging to the genus *Pfeifferella* resemble those of the diphtheria group in that they are slender rods sometimes showing branching, but they are Gram-negative. The most important species of the group is the *Pfeifferella mallei,* the cause of the disease glanders and farcy in horses and other equines.

Pfeifferella mallei

Synonyms.—*Bacillus mallei, Bacterium mallei, Mycobacterium mallei.*

The disease glanders in the horse and other solipeds is widely distributed over the surface of the earth. It is important not only because of the losses occasioned in domestic animals but also because of the danger of transmission of the disease to man.

Morphology.—The glanders bacillus is a short usually straight rod, sometimes somewhat curved, with rounded ends. It occurs rarely in short chains, usually it is single. In the stained mounts made from infected tissues the cells occur frequently in pairs. Involution forms, enlarged and clubbed cells, filamentous and branched forms are not infrequently observed. In this respect it resembles the organisms belonging to the genus *Corynebacterium*. The rods usually vary from 0.5–1μ by 1.5–5μ. They are non-motile, do not produce spores or capsules. They stain well with aniline dyes, are not acid fast and are Gram-negative.

Cultural Characters.—The organism is ordinarily found in the nasal discharges in glanders and in the pus from farcy ulcers in the horse. In order to secure the organism in pure culture, it is customary to inject intraperitoneally into a male guinea pig. Within two to four days, the organism may be isolated in pure culture from lymph glands or from the testes. The orchitis developing is quite characteristic.

The organism grows readily upon the ordinary media, particularly when they contain glycerin or blood serum. Upon agar and glycerin agar the colonies are usually circular, glistening and whitish or yellowish. Growth upon potato medium is among the most characteristic. Within two days at blood heat a yellow, honeylike, transparent growth which finally turns brownish or amber in color is developed, while the potato itself becomes a greenish-brown color.

Physiology.—The organism is aërobic and facultative anaërobic, growing best at blood heat but fairly well at lower temperatures. It is readily destroyed by heat.

Pathogenesis.—The organism is primarily an invader of the lymph glands of the horse. The first lesions are usually in the nose, producing the disease type termed glanders. When the subcutaneous lymph glands are

affected, the disease is termed farcy. The disease may be either acute or chronic. The former is more common in the ass and mule, the latter in the horse. The chronic type of disease may not show anything particularly characteristic in the early stages, but the lymph nodes become enlarged. The lesions are generally present on the nasal mucosa in the lungs and in the lymph glands. When these occur in the nose they break down, form pus and develop into chronic ulcers which eventually heal with the formation of a star-shaped scar which is quite characteristic. In farcy or cutaneous glanders nodules form below the skin, the lymph glands and ducts become swollen and appear like strings of beads. When these nodules become enlarged they may break through to the surface of the skin and ulcerate. In man the organism commonly gains entrance through a wound in the skin or by inhalation and is generally fatal.

Immunity.—Practicable methods of either active or passive immunization against glanders have not been developed, although many attempts have been made.

Diagnosis.—The disease glanders is particularly dangerous not only because of the high mortality among horses affected, but also because of its transmissibility to man. In most states in the United States animals known to have glanders must be killed and the body completely destroyed by burning. Inasmuch as the disease is difficult to recognize clinically in chronic cases, several methods of diagnosis have been worked out for this disease. It is possible to use the *agglutination test*. In general horse serum will not agglutinate glanders bacilli in dilutions of one to four hundred or more unless the animal is diseased. Usually diseased animals will show agglutination in dilutions of serum of one to two thousand. *Fixation of complement* has also been used as a satisfactory method of diagnosis of the disease. Most commonly used, however, is the so-called *mallein test*. Mallein is prepared by growing the organism

in a flask containing glycerin bouillon, then killing by heat and evaporating to about one tenth of the volume. Such glycerin extract of the glanders bacillus (mallein) when injected subcutaneously into animals suffering from glanders will cause a rise in temperature usually beginning between the fourth and eighth hour. An area usually the size of a dinner plate about the site of the injection becomes inflamed and swollen. As in tuberculosis the test is sometimes made by dropping purified mallein into the eye. In animals which react the conjunctiva becomes inflamed, the lids are swollen and a purulent discharge collects at the inner corner and on the hair below.

Transmission.—The disease is transmitted readily from one animal to another through infected foods, drinking troughs and mangers, occasionally through wounds or skin lesions. Veterinarians and horse men sometimes become infected through the skin, rarely by inhalation.

CHAPTER XXXI

SPIRAL BACTERIA—ASIATIC CHOLERA—THE GENUS VIBRIO

The organisms belonging to the genus *Vibrio* have been isolated repeatedly from water, sewage and the alimentary canal of man and animals. A few species are known which are associated with disease. One, the *Vibrio metchnikovii* produces a septicemia of fowls in Europe and the *Vibrio choleræ*, the disease Asiatic cholera in man. The latter disease is the only one which has invaded the United States and it alone will be discussed.

Vibrio choleræ

Synonyms.—*Spirillum comma, Spirillum choleræ asiaticæ, Microspira comma.*

This organism is the specific cause of the disease Asiatic cholera in man. It was first discovered by Robert Koch in 1883 who found it in the rice-water stools of patients having the disease. Apparently this disease is constantly present in certain parts of Asia, particularly in China. From this region it has spread in epidemics over Europe and United States during the past century.

Morphology.—The cholera vibrio is a short, slightly curved rod frequently known as the "comma bacillus." It is motile by means of a single polar flagellum. Elongated cells and involution forms are commonly produced in culture media. No spores and no capsules can be demonstrated. The organism is Gram-negative and stains readily with the usual aniline dyes. Isolation of the organism from the tissues of an Asiatic cholera patient is usually accom-

plished by placing fecal material in a flask of broth kept at blood heat. The organisms of various types multiply, but the cholera and other vibrios are most abundant near the surface of the medium, that is, they are attracted to the higher concentration of oxygen. A loop full of the surface film examined microscopically will usually show vibrios if they are present. The organism may then be isolated by use of an alkaline egg medium which will inhibit the growth of other types of bacteria. The organism is easily destroyed by heat. It produces acid from dextrose but no gas. Indol is formed. The *Vibrio choleræ* liquefies gelatin. Milk is not coagulated.

Pathogenesis.—The cholera vibrio is not markedly pathogenic for laboratory animals. The disease in man is acquired in much the same manner as is typhoid fever. The bacteria are excreted in the stools and the disease is transmitted only by ingestion of such material in water, milk or food.

The disease may be characterized as an extremely acute type of diarrhea. The organisms multiply in the alimentary tract, producing a poison which causes more or less desquamation of the lining membrane of the intestines. This appears in the watery stools as white particles, somewhat resembling grains of rice, whence the name rice-water stools. The disease is usually fatal.

Vaccination against the disease by the use of cultures killed by heat or considerably attenuated has been successfully practiced.

CHAPTER XXXII

PATHOGENIC FUNGI, MOLDS AND YEASTS—THE GENERA BLASTOMYCES, ASPERGILLUS, SPOROTRICHUM, TRICHOPHYTON, MICROSPORUM, ACHORION, OIDIUM.

A CONSIDERABLE number of molds and a few yeasts are known which are capable of producing disease in man or animals. For the most part these are not common but are deserving of brief mention.

PATHOGENIC YEASTS

The Genus Blastomyces.—*Blastomyces* is a generic name given to those yeasts capable of producing disease. Two species have been described. One from Europe and the United States, one from Europe and Japan, the latter, *Blastomyces farciminosus,* causing a disease of the horse closely resembling farcy, the former, *Blastomyces dermatitidis,* causing disease in man. It is probable still another disease of man, termed coccidioidal granuloma caused by a *Blastomyces* has been recorded in the United States and South America. These diseases are comparatively rare and are interesting in this connection largely because they illustrate the possibility of yeastlike organisms producing disease.

PATHOGENIC MOLDS

Genus Aspergillus.—Many species of the genus *Aspergillus* have at one time or another been reported as associated with disease in animals. One species only has been commonly isolated from such infections, the *Aspergillus fumigatus,* causing the so-called aspergillosis of birds and pneumomycosis in man and many different species of

animals. *Aspergillus fumigatus* is a common mold growing upon decaying vegetable matter such as corn. It forms in culture media greenish or bluish-green, later brownish masses. The conidiophores are abundant, short, club-shaped and the sterigmata are terminal.

Most cases of aspergillosis have been reported from birds. In these the lesions are generally located in the lungs, in the air sacs and in the hollow bones communicating with them. In man and in animals (particularly the horse) infection of the lungs is most commonly observed. The organism may spread through the blood stream or possibly through the lymph channels to other organs of the body. In the lungs the air sacs and bronchioles are filled by the growth of the fungus.

It is probable that this organism grows on decaying organic matter outside of the body. The feeding of moldy grain or fodder may give ample opportunity for infection by inhalation. This is undoubtedly especially true for chicks. It has long been known that the feeding of moldy grain to chicks frequently was followed by pneumomycosis.

Genus Sporotrichum.—Organisms belonging to the genus *Sporotrichum* are molds producing hyphæ which are usually creeping and irregularly branched. Definite conidiophores are not developed. The spores are produced on the sides or ends of short branches singly or in clusters. They are usually quite numerous, oval or spherical in shape and transparent or very light-colored. One species has been found to produce disease in both man and animals in the United States. It is the *Sporotrichum beurmanni*. It is described from multiple abscesses in man. In the horse it is known to produce one of the types of epizoötic lymphangitis. The disease is characterized in the horse by the development of nodules from the lymph glands lying under the skin. These nodules are generally spherical. They enlarge but do not open directly. Purulent material collects near

the center of the nodule. The skin above becomes thinned and softened. Serum is exuded from the surface and the hair becomes loosened and a crust holding the hair together is formed. Finally the ulcers become crater-shaped and contain a little creamy pus. The disease is not common but has been recognized at several points in the United States, particularly in Pennsylvania.

The Genus Trichophyton.—Many different species of this genus of molds have been described. Most of them were isolated from cases of disease known as herpes tonsurans and ringworm. In every case they are molds which grow into the hairs, compactly filling the interior with a mass of parallel hyphæ extending longitudinally. These may be readily seen by examining hairs from diseased areas under the microscope after immersing for a few moments in a warm solution of caustic potash. The organisms may be grown readily upon suitable cultural media. Ringworms have been described from horse, cat, cattle, sheep, swine and dog. In many of these cases the disease has been known to be transmissible to man.

The Genus Microsporum.—Species of this genus are closely related to members of the preceding genus of *Trichophyton* and like them they invade the hair. In all cases the organisms grow over the surface of the hair and encase it for several millimeters from the skin with a delicate white sheath. Four different species are known which produce skin disease in man and some seven species have been described from animals including the dog, the horse and the cat.

The Genus Achorion.—The organisms belonging to this genus likewise closely resemble the preceding genera *Trichophyton* and *Microsporum*. In man the *Achorion schönleinii* is the common cause of *favus*, a disease of the scalp characterized by the destruction of the hair and the formation of a crust or shieldlike layer over the surface. A

similar disease in mice and other rodents have been observed in Europe, in America and in Australia. This disease is transmissible to man as a skin disease. It has been observed, for example, that those who have handled sacks gnawed by mice affected with this disease contracted a skin disease or favus of the exposed parts of the body and not of the scalp. A somewhat similar disease caused by *Achorion gallinæ* is the favus in the domestic fowl. This is a disease in which the fungi form a fungous collar around the base of the feathers and may attack the head, the comb, the wattles and ear lobes. It is transmissible to other animals and to man.

CHAPTER XXXIII

THE SPIROCHETE GROUP—THE GENERA TREPONEMA AND LEPTOSPIRA, THE DISEASES SYPHILIS AND YELLOW FEVER.

The organisms which belong to this group are very slender spiral forms, usually motile by means of sinuous movements of the body. In many respects they closely resemble the protozoa and it is a disputed point as to whether they belong to this latter group or to the bacteria. The group as a whole has not been studied until recent years because of the difficulties in observation. It is only by means of the dark field illuminator and by the use of special stains that in the last two decades many of the species belonging to the group have been recognized and studied.

Many diseases of man and animals are known to be caused by organisms belonging to this group. Two will be considered here. Syphilis caused by *Treponema pallida* and yellow fever caused by *Leptospira icteroides*. In addition to the diseases just enumerated, there are the so-called relapsing fevers and tick fevers of several types, caused by organisms of this group in man. A similar disease in chickens and geese is caused by a spirochete and is transmitted from one bird into another by means of a fowl tick. Similar tick-transmitted diseases have been described in cattle, sheep and horses.

TREPONEMA PALLIDA

Synonyms.—*Spirochæta pallida, Spirillum pallidum.*

This organism, the specific cause of the disease syphilis in man, was first described by Schaudinn and Hoffman in

1905. It escaped observation by earlier investigators of the disease because of its slenderness and the difficulty experienced in getting it stained.

The disease syphilis is widely spread among civilized people. It has been known from ancient times.

Morphology.—*Treponema pallida* is a slender organism less in diameter than 0.5μ and varying in length from 4 to 20μ. In this organism the spirals are regular and vary in number from three to forty. The spirals are pointed at the tip, very possibly continued into a minute flagellum When observed in a suitable medium under the microscope, the organism is found to be actively motile. It is probable that in most cases the cells divide by transverse fission, although longitudinal division has been claimed. It does not stain well by the ordinary aniline dyes. It is best observed by use of the Giemas's stain or by impregnation of the tissues with solution of a silver salt, followed by the use of a developer as in photography.

Culture.—The organism was first secured in pure culture by Noguchi, who succeeded in growing it in medium consisting of a mixture of agar and ascitic fluid into which a bit of living tissue had been dropped. A stab culture of the organism in this medium kept under anaërobic conditions leads to the development of a hazy growth along the line of the inoculation, particularly near the tissue at the bottom of the tube.

Pathogenesis.—This organism may always be found in the primary and secondary lesions of syphilis. It is possible to reproduce the disease in other animals by inoculation of the proper tissues. In man the primary lesion usually takes the form of a hard chancre, which appears in about three weeks after infection. It usually appears on or near the external genitalia. The organism then invades neighboring lymphatics and causes enlargements of the lymph nodes usually in about six weeks after the appear-

ance of the primary lesions. The organism then apparently invades the blood stream and causes the lesions of secondary syphilis. These usually appear as localized eruptions, falling of the hair and symptoms of generalized infection, such as fever. The length of time which the secondary symptoms may last is variable and depends upon the individual infected. The disease may persist in latent or chronic form throughout life of an individual. It leads to a great variety of disorders, such as paresis (softening of the brain), degeneration and production of tumors in the liver and other organs of the body, diseases of the bones and of the blood vessels.

The disease is so important, so difficult to cure except by the most careful and painstaking of medical treatment extending over a period of years, that much study has been devoted to it. It is most frequently recognized in individuals not showing the active symptoms of the disease by the use of the complement fixation reaction or Wassermann test. Methods of satisfactorily immunizing against the disease have not been developed.

Syphilis is one of the most dreaded and loathsome of diseases. It is most frequently transmitted by coition, rarely through infected drinking vessels, toilets and by direct inoculation as in surgical work. The disease may be transmitted from mother to child, possibly even to the third generation.

Leptospira icteroides

It is probable that this organism is the specific cause of the disease yellow fever in man.

This disease is one of the most important of the tropical plagues of man and has during the warm season frequently invaded the temperate zone, particularly the Mississippi valley in the United States.

The organism is a slender, irregular spiral found in the

blood and various body organs, particularly the spleen and liver. It was first described by Noguchi in 1919.

Pathogenesis.—Yellow fever is a disease primarily of man, although Noguchi has shown that the leptospira can be injected into guinea pigs and will produce a disease resembling yellow fever. It is of particular interest because of its method of transmission. Yellow fever is not transmitted by contact, that is, it is not a contagious disease. In order to be transmitted, a mosquito belonging to the genus *Stegomyia* must suck the blood of a person who is suffering from the disease. The organism then passes through a period of incubation and probably passes a part of its life cycle in the body of the insect. Ten days later if the mosquito bites a healthy individual, it will, after the puncture of the skin, inject a sufficient number of the organisms to produce the disease. It is evident that the disease is one to be eradicated finally by proper screening, and by oiling or draining the breeding grounds of the mosquito, that is, by the eradication of the mosquito plague itself.

CHAPTER XXXIV

BACTERIA WHICH CAUSE PLANT DISEASES—THE GENERA ERWINIA, PSEUDOMONAS AND LACTOBACILLUS

In recent years it has come to be recognized that a considerable number of plant diseases are due to the activity of pathogenic bacteria. Practically all of the forms producing disease in plants belong to two genera, *Erwinia* and *Pseudomonas.*

The genus *Pseudomonas* is characterized by being made up of rod-shaped, short organisms, usually motile by means of polar flagella, rarely nonmotile. They are aërobic and facultative anaërobic. Frequently they are capable of liquefying gelatine; they do not produce spores. The Gram stain is variable, usually negative; usually there is comparatively slight fermentation of carbohydrates. Many of the species produce a water soluble pigment, green, blue, purple, brown, etc. Among the plant pathogens, a nondiffusible yellow pigment is frequently produced. Some species are white.

The genus *Erwinia* is very closely related to the genus *Bacterium,* that is, to the organisms of the colon typhoid series of bacteria which have already been discussed. All of them are plant pathogens. The growth upon culture media is usually whitish and often slimy. Acid is usually formed in certain carbohydrate media and occasionally gas. The cells are rod-shaped, without endospores, Gram-negative and either motile by means of peritrichic flagella or occasionally nonmotile.

Erwinia amylovora

Synonyms.—*Bacillus amylovorus.*

This organism is the cause of a specific disease, pear

blight, so destructive to the pear and affecting also others of the pomaceous fruits such as the apple. The disease is widely spread over the United States.

Morphology.—The organism is a short rod, motile by means of flagella; from 0.5 to 1μ in diameter and 1 to 1.8μ in length. It stains readily with the ordinary aniline dyes and is Gram-negative.

Cultural Characters.—The growth upon artificial media is quite uniformly good; upon agar it produces a grayish white, buttery growth. Slight liquefaction in gelatin occurs after several weeks. There is some production of acid from dextrose but no gas is developed.

Pathogenesis.—It is believed that this organism is transferred from plant to plant by bees, by plant lice and by other insects. The leaves turn brownish or black, the stem turns black and the young twigs show a blackened bark. The name "fire blight" is often given to this type of infection because the infected branches have a scorched appearance. It may also invade the larger branches and trunk of the tree, producing the so-called body blight.

The disease is combated by pruning out all diseased wood. Care must be used to remove all infected twigs and branches, the cutting should take place some distance below the visible infection inasmuch as the bacteria ordinarily, at least, early in the season, are a considerable distance in advance of the blackened area. The wood cut away should be burned. Instruments used in cutting out wood should be carefully disinfected, otherwise they may aid in spreading the disease from tree to tree.

Erwinia solanacearum

Synonym.—*Bacillus solanacearum.*

This organism is the cause of a wilt attacking many plants belonging to the family *Solanaceæ*, particularly the tomato, egg plant, potato and tobacco.

Morphology.—The organism is about $0.5 \times 1.5\mu$, motile by means of peritrichous flagella; stains readily with ordinary aniline dyes and is Gram-negative.

Cultural Characters.—In broth there is abundant dirty white sediment, the reaction usually becomes alkaline. Milk is rendered alkaline, casein is dissolved without precipitation. On agar the growth is smooth, slightly viscous, first whitish, then yellowish and finally brown. Gelatin shows little or no liquefaction; on potato the growth is white and later brownish to black on a browned medium. Neither acid nor gas are produced from the carbohydrates.

Pathogenesis.—The disease is characterized by sudden wilting or shriveling of the foliage, the softer parts of the plant also wilt rapidly. The fibrovascular bundles of the stem become stained brownish and microscopically are found to be filled with bacteria. The wilting is most evident in the young plants. Older woody plants may turn yellow and die without wilting. The organism may invade the parenchymatous tissues and cause rotting. The potato tubers infected with the organism show brownish discoloration in the ring of fibrovascular bundles.

Erwinia tracheiphila

Synonym.—*Bacillus tracheiphilus.*

This organism is the specific cause of a wilt disease of cucurbits, particularly of cucumber, squash, cantaloupe and pumpkin. In this disease the foliage shows a sudden wilting and shriveling because the fibrovascular bundles are stopped by the large masses of white, sticky, rod-shaped organisms.

Morphology.—The organism is a rod, motile by means of peritrichous flagella, about 0.5μ in diameter and from $1.2–2.5\mu$ in length. It stains readily and is Gram-negative.

It is probable that the disease is spread largely through insects, particularly by the cucumber beetle. The organism

gaining entrance into a leaf causes first a localized wilted area, the organisms invade the larger vessels of the fibrovascular bundles and the entire leaf wilts. Upon invasion of the main stem the organisms spread rapidly, the vessels are occluded and the entire plant wilts. When the stem is cut the sticky white bacterial mass oozes from the cut bundles, and when touched frequently shows its glutinous character by drawing out in tiny threads.

Erwinia carotovora

Synonym.—*Bacillus carotovorus.*

This organism causes a soft rot of a considerable number of root crops, particularly carrots and turnips.

Morphology.—The organism is about $.8 \times 2.0 \mu$, motile by means of peritrichous flagella. The other characters are those common to the genus.

Culture and Physiology.—The organism grows readily upon the usual culture media, generally abundantly to luxuriantly. On agar the growth is smooth, glistening and white. Gelatin is liquefied relatively rapidly. A scum forms in broth. Milk is coagulated and slowly digested. Small amounts of gas and acid are produced in dextrose, lactose and saccharose broth and acid from glycerol.

Pathogenesis.—This organism usually gains access to the tissues through a wound or injury. It produces an enzyme pectinase capable of dissolving the middle lamella of the cells. The cells then fall apart and are themselves finally invaded and destroyed. The disease is, therefore, a typical soft rot.

Erwinia melonis

Synonym.—*Bacillus melonis.*

This is the cause of a soft rot of the fruit of muskmelons.

Morphology, Culture and Physiology.—It is much like the preceding organisms and typical of the genus. Acid and gas production are peculiar in that no gas is produced in sugar broth, though growth occurs and acid develops, while some gas is said to develop in asparagin broth and in milk, the gas consisting of carbon dioxide.

Pathogenesis.—The organism apparently invades the melon from the soil through cracks or injuries. Like the preceding organism it is capable of dissolving the middle lamella and of producing thereby a typical soft rot.

Erwinia phytophthora

Synonym.—*Bacillus phytophthorus.*

This organism has been described from Europe and America as the cause of the basal stem rot of the potato. It also causes rotting of the tubers. It closely resembles the other members of the genus in its morphology and culture.

Lactobacillus teutlius

Synonym.—*Bacterium teutlium.*

This organism is described as the specific cause of a soft rot of the sugar beet. It is apparently culturally and morphologically most closely related to the lactic acid bacteria, particularly those members of the genus *Lactobacillus* most important in the production of acid in the fermentation of ensilage.

Morphology.—The organism is a nonmotile, Gram-positive rod, $.8 \times 1.5\mu$, without capsules or spores and staining readily with the ordinary aniline dyes.

Cultural and Physiological Characters.—On media not containing sugar, growth is usually scant, slow, white and not viscous. On agar it tends to grow into and penetrate the medium. In the presence of cane sugar the development is much more rapid and abundant, becoming watery

or viscid. Gelatin is not liquefied. No growth occurs on potato. There is no gas, but acid, from dextrose and saccharose, and no visible growth in milk.

Pathogenesis.—The bacteria apparently gain access to the root through injuries, particularly those produced by nematodes. The portion of the beet below ground decays and becomes filled with areas containing an acid, viscous or slimy liquid.

Pseudomonas campestris

Synonym.—*Bacillus campestris.*

The black rot of cabbage and related cruciferous plants and its causal organisms was first described by Pammel in Iowa, later it was found in many other portions of the United States and of Europe.

Morphology.—The organism is a Gram-negative rod, slender, 0.4–0.5 $\times$ 0.7–3.0μ. In young cultures, the cells are motile by means of a single polar flagellum. No capsules and no spores are produced.

Cultural and Physiological Characters.—Growth is abundant upon most culture media. Cultures on solid media are markedly yellow, older cultures are brownish. Growth is moist, slimy and shining. Milk is made alkaline. Gelatin is liquefied slowly. No gas is produced from carbohydrates. Indol is developed.

Pathogenesis.—The bacteria apparently usually enter the leaf through the marginal water pores. The fibrovascular bundles are invaded and turn dark. Gradually most of the surrounding tissues are destroyed or disorganized. The disease has proved very destructive in many localities.

Pseudomonas tumefaciens

Synonym.—*Bacterium tumefaciens.*

This organism is the specific cause of crown gall, soft gall and hairy root in most of the species of orchard fruits,

grapes, raspberries, blackberries, the Paris daisy and many other plants.

Morphology and Culture.—The organism is a Gram-negative rod, motile by 1 to 3 polar flagella. Growth differs from most of the other members of the genus *Pseudomonas* in that it is white instead of yellow. Milk is made alkaline. Gelatine is not liquefied. No gas is produced from carbohydrates.

Pathogenesis.—The organisms gain access to the plant tissues through a wound. It is most common at the juncture of graft and scion in grafted trees. If the meristematic tissues giving rise to medullary rays or soft tissues are invaded there is an enormous proliferation of cells, and a soft gall is formed which usually ultimately disintegrates. It the tissues invaded are those which give rise to the woody tissues, the tumor developed is hard. Frequently the roots are stimulated to abnormal development producing masses which give rise to the name hairy root. Artificial inoculation gives rise in suitable host plants to the development of primary and secondary tumors. In many respects the tumor in the plant simulates a cancer. The disease results in severe loss to nursery men. Certain apples in particular are very difficult to secure free from crown gall. It is probable that infection occurs commonly at the time of grafting. Trees grown from galled plants are frequently defective, weakened or stunted in their development.

Pseudomonas stewarti

Synonym.—*Bacterium stewarti.*

This organism is the cause of a wilt of sweet corn. It has been found in several sections of the United States, though first reported from Long Island.

The organism is a typical yellow pseudomonad in morphology, culture and physiology. It invades the fibro-vascular bundles, cutting off the supply of water, in con-

sequence of which there is prompt wilting of the leaves. Yellow, slimy masses of bacteria ooze from the surface of the cut bundles, which are made yellow. Apparently the bacteria may enter the plant through the roots or the water pores and stomata.

Pseudomonas jugalandis

This organism causes a serious blight of English walnut in parts of California. It is a typical yellow pseudomonad. It produces discolored or black cankers on the nuts, injuring them commercially. It also attacks the young shoots.

Pseudomonas medicaginis

This organism is the cause of stem blight of alfalfa, a disease of the first crop of this plant in certain parts of the western United States. It is described as producing watery, semitransparent yellowish or blue-green appearance on one side of the stem. The bacterial slime oozes from the diseased area. This dries, giving the stems a varnished appearance. Occasionally the crown of the plant may be involved. Apparently the bacteria gain entrance through cracks caused by freezing and thawing in the spring of the year.

The organism differs from most of the other pseudomonads pathogenic for plants in that it produces a fluorescent green pigment when grown on agar.

CHAPTER XXXV

PROTOZOA CAUSING DISEASE—THE GENERA BABESIA, PLASMODIUM, ENTAMŒBA, COCCIDIUM AND TRYPANOSOMA.

The protozoa are the one-celled organisms belonging to the animal kingdom; they occupy the same general relationship to the higher forms of animals as the bacteria do to the higher forms of plants. They live throughout their lives as single celled individuals or in colonies of single cells. That is to say, the cells do not unite at any time to form tissues or organs and the various species never become multicellular. It was previously noted that there are many intergradations between the bacteria and the protozoa, such as *Treponema.*

The protozoa in general, though they are single-celled, are much more complex in structure and in life histories than are the bacteria. The cells of many forms have been greatly modified and different portions of the cell differentiated for particular purposes. These specialized parts are termed the *organella* of the cell.

Classification of the Protozoa.—All the members of the protozoa may be grouped into two great subdivisions. The first of these includes those forms which can move about either by means of pseudopodia or flagella, the second including those motile by means of numerous cilia. Cilia differ from flagella in that they are shorter, more numerous, generally blunt, and move the organism about by striking the water in unison, resembling oars in their action. The first subdivision is termed *Plasmodroma,* the second subdivision *Ciliophora.* The group *Plasmodroma* is divided into three classes: 1. *Mastigophora* includes those forms

which are motile by means of flagella; 2. *Rhizopoda* includes those motile by means of pseudopodia; 3. *Sporozoa* includes forms that are variously motile and greatly reduced through parasitism. At some time in their life apparently they all produce numerous spores.

None of the group of *Ciliophora* will be considered.

One genus belonging to the *Mastigophora, Trypanosoma,* includes organisms which are the cause of a variety of diseases in man and animals. One genus belonging to *Rhizopoda, Entamœba,* is the cause of amebic dysentery in man, and three genera belonging to the *Sporozoa, Babesia, Plasmodium* and *Eimeria,* cause Texas fever in cattle, malaria in man and coccidiosis in fowls respectively.

Genus Trypanosoma

A trypanosome is a protozoan possessing an elongated cell usually more or less spindle-shaped, occasionally almost as broad as long but tapering at the ends. Practically all of the forms which are of importance from the standpoint of disease production are longer than broad when they are observed in the blood. The posterior end of the cells terminate in a flagellum, the length depending upon the species. Along one side of the cell extending from one end to the other is a thin membrane. The flagellum extends along the membrane and forms its outer edge. The flagellum finally enters the cells near the anterior end and terminates in a granule (the blepharoplast) which may stain readily when treated with aniline dyes.

The cells multiply by a splitting first of the blepharoplast, later followed by a division of the nucleus and a longitudinal splitting of the cell. It is probable that some at least, of the trypanosomes pass through a regular life cycle. Some have been successfully cultivated in culture media by mixing rabbit blood in melted agar and preparing a slant in a test tube. The water of condensation is planted

with a small amount of blood containing the trypanosomes to be cultivated. They multiply in this water of condensation in some cases quite abundantly.

Disease Produced.—The only disease occurring in the United States caused by a trypanosome is the *dourine* of the horse, sometimes termed *horse syphilis*. The disease is also known from various parts of western and southern Europe and northern Africa. It has been introduced at various times with imported animals and cases have been known from several of the states in the Mississippi Valley and in Canada. Animals which are infected gradually become emaciated and swellings appear upon the genitals and whitish chalklike areas (plaques) develop in the skin and mucosa of the external genitalia. The disease is usually chronic in form and recovery does not frequently occur. The disease is probably transmitted by the stallion. It is claimed by some authors that the disease can also be transmitted by means of the stable fly but this probably does not occur frequently.

Disease produced by trypanosomes (so-called trypanosomiases) are of great importance in tropical countries and in the southern hemisphere. There are large areas in Africa, for example, where it is quite impossible to keep horses because of the presence of the so-called *nagana* or tsetse fly disease. The organism is found among certain of the wild animals and is inoculated into the horse by the bite of the tsetse, a fly somewhat resembling a horsefly. In South America a disease known as *mal de caderas* or rump evil of the horse is known. In this disease there is progressive emaciation of the horse, the hind quarters become weak, the horse when walking will scarcely raise the hoofs off the ground. The method of transmission is not certainly known. Another trypanosome causes the Gambian horse sickness in Africa, another the so-called baleri of horses, cattle, sheep and goats in the French Congo.

Human trypanosomiasia or sleeping sickness is caused by the *Trypanosoma gambiense.* This disease has made uninhabitable a considerable section of north, central and eastern Africa. It is transmitted by means of one of the tsetse flies. It is probable that the organism is present in certain of the wild animals in this region. In the first stages of the disease the organisms can be found in the blood. Later pains in the back develop and the patient becomes drowsy, sinks into a coma from which he cannot be readily aroused and finally dies. The disease is chronic; sometimes several years elapse before it proves finally fatal.

A disease known as *murrina* in the horse in Panama, Venezuela, and Central America has also been traced to a *Trypanosoma.*

The Genus Entamœba

An ameba which is found as a normal inhabitant of the intestinal tract in man is known as *Entamœba coli.* An organism somewhat resembling it and causing so-called amebic dysentery is termed *Entamœba histolytica.* The genus *Entamœba* differs from *Amœba* in the absence of contractile vacuole and by the formation of multinucleated cysts.

The *Entamœba histolytica,* the cause of amebic dysentery may be observed in the stools of those who are suffering from the disease as a mass of protoplasm without definite cell wall and possessing a definite nucleus. It moves about by means of the protrusion of blunt pseudopodia. It is either colorless or slightly tinged with green due to the presence of hemoglobin from blood corpuscles which have been engulfed. The inner portion of the protoplasm (endoplasm) and the outer portion (ectoplasm) may be readily differentiated in that the latter is hyaline, glasslike and more refractive. Reproduction of the organism is accomplished in two ways. In the first method the nucleus fragments into a number of nuclei which gradually collect under

the ectoplasm, develop into mature nuclei and, together with some protoplasm, are finally pinched off as spores or buds. The disease is apparently contracted by drinking water or eating food which has been fouled by the excretions of those who harbor the organisms. The disease itself is a chronic dysentery, frequently associated with intestinal ulceration and not infrequently with abscesses in the liver.

The disease blackhead in turkeys is due to an organism named *Amœba meleagridis*. This organism is abundant in the cæca of the bird. The characteristic lesions are thickening and ulceration of the walls of the cæca. Apparently it is a secondary parasite and occurs in birds in which the cæcal walls have been injured by the attack of the intestinal worm *Heterakis*. It kills young birds rapidly and in some sections of the country has made the raising of turkeys almost impossible.

The Genus Babesia

The organisms belonging to this genus are parasites in the blood corpuscles of various species of animals. They cause the diseases equine biliary fever, a disease noted from India and South Africa in horses, hemoglobinuria or red water in sheep in Roumania, jaundice of the dog in various parts of Asia, Europe, Africa and possibly in the United States. The most important species is the *Babesia bigemina* causing red water, Texas fever or tick fever in cattle.

Babesia bigemina.—This organism was discovered by Theobald Smith in 1889 during his work with Texas fever in cattle. The disease is known from the southern United States, Australia, South America, Europe, India, Philippines and Africa. When blood from animals affected with the disease is examined microscopically, red blood cells are in part found to be invaded by the protozoan. The cells of the parasite are usually pear-shaped, one end being rounded and the other somewhat pointed. Usually two cells are

found within a single blood corpuscle. The organisms are from 0.5–2$\mu \times$ 2–4μ. The disease in cattle is characterized by fever and by the presence of hemoglobin in the urine. There is considerable destruction of the red blood corpuscles. In acute cases death often intervenes in from 5 to 8 days after the first symptoms. Most animals recover but usually harbor in their bodies organisms for a long period of time, though showing no symptoms of the disease. The injection of blood from immune animals is widely practiced as a method of vaccination in nonimmune cattle. It results in a mild infection which immunizes against the severe type when transmitted in the regular manner.

The disease is normally transmitted only by the bite of infected cattle ticks. The mature female tick gorges on the blood of an animal, then falls to the ground and after a time the eggs are laid. These hatch after from 19 days to 5 or 6 months, depending upon conditions, whereupon the young ticks (called seed ticks) crawl up the stems of grass and shrubs. They attempt to attach themselves to passing animals. If they do not succeed, they die of starvation. Ticks that have come from a mother that has fed herself on blood from an infected animal are themselves capable of transmitting the disease to the animal whose blood they in turn suck.

The disease is gradually being eradicated in the United States. Within the last few years the line which marks the northern border of the tick quarantine district has been moved south until some of the southern states which formerly were in the quarantine district have been relieved entirely. Within a few years the disease will probably have been completely eradicated.

The Genus Plasmodium

The organisms belonging to the genus *Plasmodium* are responsible for the disease malaria in man. Three species,

at least, are known. They differ in certain characteristics of morphology and life cycle but closely resemble each other on the whole. These organisms are *Plasmodium vivax,* the cause of tertian malaria in man, *Plasmodium malariæ,* the cause of quartan malaria, and *Plasmodium falciparum,* the cause of malignant or tropical malaria.

The organism is present in the blood stream of individuals infected with the disease. It attaches itself to and probably penetrates the red blood corpuscles and develops in the interior of the cell so the corpuscle is practically entirely filled with the organism, causing it to become somewhat swollen. The organism then segments to form a rosette of bodies which round off to form small spores called *merozoites.* These become free by the breaking to pieces of the red blood cell and in turn attach themselvs to other cells and begin the cycle again. This may be repeated over and over. It is termed the asexual part or phase of the life cycle. The organism may be taken in with blood from a patient by a mosquito belonging to the genus *Anopheles* (or to a related genus), whereupon it passes through several distinct stages of development. In the body of the mosquito two types of cells are formed from the spores taken in with the blood, male cells and female cells, called respectively *microgametes* and *macrogametes.* Fertilization of the macrogamete by fusion with a microgamete occurs, the resultant cell being termed a fertilized egg or oökinete. This makes its way by boring into the gut wall of the mosquito, where it becomes encysted and enlarges to form a tumorlike swelling. The contents then break up to form a considerable number of spherical bodies known as *sporoblasts,* which in turn subdivide and produce within themselves great numbers of delicate filaments called *sporozoites.* These are eventually freed by breaking of the cyst and pass out into the body cavity of the mosquito. Some of them eventually make their way to the salivary or poison gland

of the mosquito, when they are inoculated into the person bitten by the mosquito. It requires from 8 to 10 days from the time the mosquito bites a person having the malarial parasite in his blood until the mosquito can in turn transmit the disease to another individual.

The disease is commonly known as *ague* or *chills and fever*. It is characterized by chills followed by fever at intervals of 48 hours. During the period between attacks of chill and fever the temperature is normal. It is interesting to note that the chill and fever occur at the time when the merozoites are breaking out of the red cells and attacking new red blood corpuscles.

The description given applies to *Plasmodium vivax*. In quartan malaria the interval between paroxysms of chills and fever is 72 instead of 48 hours. In the tropical type of malaria, two forms are known, one in which the life cycle is completed in 24 hours and fever consequently appears daily, and a tertian type in which the fever occurs every 48 hours.

Inasmuch as the disease can be transmitted only by the bite of infected mosquitoes, it can be eliminated only by preventing the breeding of these insects or by preventing their transmitting the germ from infected individuals to those who do not have the disease. Since mosquitoes breed in stagnant water the disease has been eradicated in many localities by drainage, by the use of oil on the surface of water and by the introduction of fish which feed upon the larvæ of the mosquito.

The Genus Eimeria (Coccidium)

The organisms belonging to this group that infect animals or man are intestinal parasites. The adult cells are oval or spherical and not motile. The life history is quite complex and varies with the different species. The most important form in North America probably is the *Eimeria*

(*Coccidium*) *avium*, the cause of coccidiosis in domestic fowls and in birds. The disease is widely distributed over the United States and Europe, but has not been extensively studied. The organism is taken into the body with food in the form of a cyst which ruptures and allows the escape of spindle-shaped protozoa. These burrow into the epithelial cells of the intestinal walls or other membranes as in the cæca. Upon entering a cell the spore rounds up into a sphere and grows rapidly in size. The nucleus of this cell breaks into a number of pieces and the protoplasm itself divides into a considerable number of spindle-shaped cells. These break away from the mother cell and in turn invade new cells. This procedure may be repeated several times. Eventually some of them develop as sexual reproductive cells. Some of these are larger than the remainder, the so-called macrogametes or eggs and the microgametocytes which resemble macrogametes at first but divide into a large number of very slender threadlike cells termed microgametes. These are freed by the rupture of the cell and the macrogametes are fertilized by the microgametes to form an oöcyte. This continues to enlarge and secretes a firm wall (becomes encysted). When completely mature, the contents of the cyst divide to form several spherical cells or bodies. These elongate and become spindle-shaped, and in each, two small, slender spores develop. When the cyst is mature it is passed out with the feces of the bird. If these cysts come in contact with food, they may be ingested by a suitable host, whereupon the cycle begins again.

Organisms somewhat resembling the form described are known to produce coccidiosis in the rabbit, in cattle, in sheep, and in the dog and cat.

CHAPTER XXXVI

INFECTIOUS DISEASES WHOSE CAUSAL ORGANISMS ARE NOT CERTAINLY KNOWN

A CONSIDERABLE number of diseases regarded as infectious are known in which the causal organisms have not been discovered or are not certainly known. It may be asked why should a disease be termed infectious, that is, caused by some organism, when the organism itself is not known? How can one be sure that an organism is responsible for a disease before such has been proved to be the cause? The reasons may be summarized as follows: All the diseases discussed are more or less contagious, that is, more or less readily transmitted from one individual to another. It is evident that something must actually pass from one individual to the next. This something is termed a *virus*. In many of the cases the cause originally described as a virus has been discovered, studied and proved to be a specific microörganism. It is assumed that the virus in all cases is a microörganism of some kind.

The reasons why the causal organisms have not been isolated and described for all diseases listed below can scarcely be determined in advance. Probably in some cases the organism will not grow in any of the media which have been tried; in other cases the microscopic examination of the lesions of the disease show bodies, which may or may not be microörganisms, but which have not been cultivated as yet. In other cases the organism, at least at certain stages in its life cycle, may be ultramicroscopic. It is possible that

in other cases suitable methods of staining or demonstration have not yet been developed.

Several of the diseases discussed here below are exanthemata, that is, they are marked by an eruption of the skin. Such, for example, are the poxes (smallpox, chicken pox and the poxes of the various animals, scarlet fever and typhus fever). The diseases which come under the category of those whose causes are not certainly known are the following: Smallpox, cowpox, sheep pox, chicken pox, horsepox, measles of various kinds, scarlet fever, infantile paralysis, foot and mouth disease, rabies, hog cholera, influenza, swamp fever, typhus fever and trench fever.

Smallpox

The disease smallpox in man has been described in medical literature for at least a thousand years. It is also known as *variola*. It is among the most contagious of human diseases and can be transmitted to some of the lower animals. Particularly characteristic of the disease are the skin eruptions which develop as vesicles and finally contain pus. These, upon healing, develop scars of variable extent. Smallpox has an incubation period of about 12 days.

The disease is of particular interest because it was the first for which a practicable method of vaccination was developed. It was shown by Edward Jenner in 1798 that vaccination by means of material taken from lesions of cowpox in cattle will result in an immunity in man to smallpox. Naturally the question at once arises, are cowpox and smallpox identical, that is, caused by the same organism. There is good reason to believe that they are. Several investigators have found it possible to develop the disease cowpox in calves by inoculation with the virus of smallpox secured from human cases. Apparently this is not readily accomplished, however, showing that there is some difference in

the microörganisms concerned. The lesions produced by the two diseases are very similar and it has been found possible in monkeys to immunize against smallpox by inoculating with cowpox and against cowpox by immunizing with smallpox.

Smallpox vaccine is usually prepared from heifer calves from 2 to 4 months old. They are inspected carefully to see that they are not diseased. The animal to be used for the preparation of vaccine is carefully washed and cleaned. The posterior portion of the abdomen and the inside of the thighs are carefully shaved and washed. Shallow cuts are then made both longitudinally and crosswise over a large area. Vaccine from a suitable source is then rubbed into each one of these incisions. The area is then covered with cotton to protect from dirt. The vaccine is usually ready for collection in five or six days. The inoculated area is then cleaned and the hardened crust removed. The soft material remaining is scraped off and placed in a suitable container. It is then mixed with glycerin (50 per cent), and triturated in a mortar until it is homogeneous. One-half per cent of phenol is also added. The vaccine is ready after standing for some weeks. Any bacteria which have gained access during preparation have usually been destroyed and the virus is ready to be used as a vaccine after having been tested for potency. It may be then used for vaccination in man. At a result of the widespread use of vaccination, the disease has lost most of its terrors for civilized people. Usually the immunity resulting from vaccination lasts for some years.

Stained sections from the lesions characteristic of smallpox show peculiar cell-like inclusions. These are termed the vaccine bodies. Some have contended that these vaccine bodies are artefacts, others that they are living organisms, probably protozoan in nature. Some investigators have even worked out the probable life cycle of the organism.

Chicken Pox

The organism responsible for the disease chicken pox has not been determined. It is probably related to the organism which causes smallpox, although, of course, entirely distinct. The eruptions of chicken pox are more superficial and do not result in the pitting and scars so characteristic of smallpox.

Measles

Three, possibly four, distinct types of measles are known. The most common are the so-called little red measles and the German measles. The causal organisms of these diseases are not known. They are highly contagious. The incubation period is usually 9 or 10 days. The symptoms begin with headache, fever and sore throat or "cold in the head," with a rash which develops about the fourth day. Characteristic spots appear in the mucous membrane of the mouth. Methods of immunization against the disease are not known. The disease is passed on by more or less direct contact.

Scarlet Fever

This is an acute, highly contagious disease showing a diffuse erythematous skin eruption. There is usually a catarrhal, croupous or a gangrenous inflammation of the throat, and fever. The causal organism is not known. Streptococci are very common as secondary invaders to the disease and many of the sequelæ of the disease are due to these organisms.

Infantile Paralysis

This disease, known also as acute anterior poliomyelitis or the Heine-Medin disease, has been known for nearly a hundred years in Europe and has caused several epidemics in recent years in the United States. Flexner and Lewis in 1909, showed the causal organism to be a filterable virus,

very probably ultramicroscopic. The disease is one in which the spinal cord is primarily infected, leading to a total or partial paralysis of the limbs. The disease has been transmitted in typical form to monkeys. It is probable that the disease is spread by ingestion of infected materials. Several organisms have been reported as commonly associated with the disease, but adequate proof as to their actual causal relationship is as yet lacking.

Foot and Mouth Disease

This is an acute contagious disease infecting cattle, sheep, goats, swine, deer, occasionally horses, dogs and cats. It may be transmitted to man. It has been known for over a century from Europe and has caused several epizoötics in the United States. Certain investigators have claimed the causal organism to be a protozoan which they have named *Aphthomonas infestans.* Stauffacher claims to have succeeded in cultivating the organisms and that the disease may be transmitted by the use of pure cultures. The report lacks adequate corroboration at the present time.

The disease is characterized by an acute fever, the appearance of vesicular eruptions in the membranes of the mouth and on the feet, particularly between the toes. The disease is usually not fatal, but is so highly contagious and leads to such losses in milk, that it is among the most dreaded of the diseases of cattle. Cases have been reported in man due to the drinking of milk coming from diseased animals.

In parts of Europe vaccination has been extensively practiced with the idea of producing the disease and "having it over with." Apparently the organisms gain entrance through contact of healthy animals with saliva or other excretions of infected animals. The disease has been dealt with in the United States almost exclusively by the stamping-out process. There have been some instances of transmission of the disease through the use of blood from hogs

hyperimmunized against hog cholera, infected accidentally with the foot and mouth disease, the serum being used for the immunization of healthy swine. One of the outbreaks in the United States was due to the introduction of the virus with smallpox vaccine from calves infected with the disease.

Rabies or Hydrophobia

This disease is known as hydrophobia in man and rabies or lyssa in animals. The causal organism of the disease is questionable. It has been claimed by a group of investigators headed by Negri that certain bodies which may be demonstrated in the larger ganglion cells of the Ammon's horns of the brain are the specific organisms capable of causing the disease. These have been named the Negri bodies. These bodies are so constantly present in the brains of animals having the disease that they are regarded as diagnostic. Whether they are specific protozoa or degeneration products of brain cells has not been definitely proved.

Rabies is a disease primarily of dogs and closely related carnivora, transmissible to a wide variety of animals and to man through direct inoculation.

The virus enters the body through a wound, usually the bite of a rabid animal. After an incubation period of several weeks the symptoms of the disease are manifested. Apparently this time is required for the causal organism to pass from the site of initial infection to the central nervous system.

The disease is of particular historic importance as it was the first of the diseases of man in which vaccination proved effective, with the exception of smallpox. The Pasteur treatment is carried out by vaccination with an attenuated virus. Tests made upon experimental rabbits have shown that virus secured from different diseased animals, the so-called "street virus" is decidedly variable in its ability to produce disease. By passage through a number of

rabbits consecutively, the virulence is increased until it will kill rabbits regularly in from six to seven days. This is then termed a "fixed" virus. The vaccine is prepared by removing under aseptic precautions, the spinal cord from a rabbit dead as a result of an injection of fixed virus. This is carefully dried in a desiccator at a constant temperature of 23° C. for a period of two weeks. The vaccine is an emulsion of this cord in physiological salt solution. The drying has so attenuated the virus that it may be injected with impunity into man or animals. Later other injections are made with emulsions from spinal cord dried for a shorter period of time. Inasmuch as the disease has a relatively long incubation period, the time elapsing between the bite of a rabid dog and the normal appearance of symptoms may be used for immunization. This method, first used by Pasteur, has proved highly successful.

Hog Cholera

This disease of swine is one of the most important affecting the live stock industry. There was much difficulty in the earlier study of the disease due to confusion with other swine diseases, and particularly because from 1885, when Salmon and Smith described the organism *Bacterium cholerœ suis* as the cause of the disease, until 1904 the scientific world labored under a misunderstanding of the cause. In the latter year De Schweinitz and Dorset demonstrated that the typical hog cholera in the United States is due to a filterable virus. This discovery led to the development of satisfactory methods for immunization against the disease. The organism which causes hog cholera is classed as a filterable virus, inasmuch as it will pass readily through porcelain filters. The organism in the blood serum of hogs will remain virulent for many weeks. It may be destroyed by disinfectants, but is relatively resistant.

Hog cholera usually manifests itself as an acute disease,

occasionally assuming a chronic form. *Post mortem* examination of acute cases usually show congestion and acute swelling of the internal organs and hemorrhages on the serous and mucous membranes and not infrequently a serous transudate in the pericardium. In the chronic type necrotic and ulcerated areas are commonly found in the intestines, frequently associated with pneumonia in the lungs. It has not proved possible to produce the disease in animals other than swine. The organism *Bacterium cholerœ suis* is undoubtedly an important secondary invader in the disease and possibly may in some cases have the ability to produce a choleralike disease in the entire absence of the virus.

The work of Dorset, MacBryde and Niles in the United States showed that the blood serum from a hog that has recovered from hog cholera will confer some degree of immunity when injected into susceptible animals. This fact has been made use of in the development of a practicable method of prevention of the disease. An animal is first secured that has already been immunized or has recovered spontaneously from hog cholera. This animal is hyperimmunized by injection of blood from a so-called virus pig, that is, one suffering from acute attack of hog cholera as a result of the injection of virus. Usually about 5 cubic centimeters of this defibrinated virulent blood is injected for each pound of body weight of the animal to be immunized. Some days later this animal is bled either from the carotid or by cutting off the tip of the tail. The blood thus secured is defibrinated and preserved by the addition of one-half per cent of phenol. The animal may again be hyperimmunized and bled several times; eventually all of the blood is removed from the body. The potency of the serum thus secured is tested upon young pigs usually weighing from 50 to 60 pounds. A serum sufficiently potent should in a dose of 15 cubic centimeters or less, protect

such an animal when injected with 2 cubic centimeters of virulent blood.

In practice the hog cholera antiserum is used either for conferring a temporary passive immunity or combined with the injection of virus for the conferring of an active immunity. In the latter method, the antiserum together with the virulent blood are injected into the animal to be immunized. The immunity thus secured is relatively permanent, while immunity secured by the injection of anti-hog cholera serum alone is relatively temporary, lasting only from 4 to 6 weeks. As a result of the utilization of this method the losses from hog cholera have been greatly reduced.

Influenza

The disease influenza is an acute infection of man which has swept in a great pandemic around the world in recent years. The ordinary form shows an onset of severe headache, accompanied by pains and aches in the back, by fever and by general prostration. The fever continues in such cases from 3 to 5 days and finally leaves the patient exhausted.

Pfeiffer in 1892 described an organism which he believed to be the cause of the disease influenza. This has been generally assumed to be the cause until investigation in the past few years failed to prove any causal relationship between this organism and the disease. While much work has been done upon the disease, the causal organism has not yet been isolated with certainty.

The disease is important not only in itself but because it is followed in a considerable proportion of cases by severe and frequently fatal pneumonia.

Swamp Fever or Infectious Anemia

This disease has been known for many years in Europe and more recently has been studied in North America. It

is a disease of the horse caused by a filterable virus found in the blood, in the urine and the feces of infected animals. Blood sera from animals showing symptoms of the disease inoculated into susceptible individuals produce the disease after 5 to 9 days or more. The first symptom is fever. The disease may be characterized as an acute or chronic anemia. There is a great destruction of blood cells. Together with this there is degeneration of kidneys and liver and changes in the blood vessels. The spleen becomes enlarged and frequently degenerates and the bone marrow shows profound degeneration. The disease generally proves fatal. Methods of transmission of the disease have not been satisfactorily worked out.

Typhus Fever

This is an acute infectious disease of man. It has been recorded in the United States under the name, Brill's disease in mild form in the city of New York, and as tabardillo in Mexico. The disease is transmitted from man to man by means of the body louse, occasionally by the head louse. The disease may also be transmitted to the monkey.

SECTION VI

SANITARY BACTERIOLOGY

CHAPTER XXXVII

BACTERIOLOGY OF WATER AND SEWAGE—SEWAGE DISPOSAL

Bacteria are normally present in most natural waters. Occasionally springs or wells may be found from which the water is practically sterile, but these are uncommon. Unpolluted waters from various sources, therefore, may be regarded as having each its characteristic flora.

Many distinct kinds of bacteria have been recorded from natural waters. Frequently the pigment-producing forms are relatively abundant, particularly the chromogenic micrococci and sarcinæ, yellow, orange and red.

The number of bacteria normally present per cubic centimeter in water varies greatly with the source and the conditions under which the water has been kept. It has already been noted that occasionally waters may be found which are practically sterile. At the other extreme we may find waters such as those heavily contaminated with sewage in which bacteria are present by the hundreds of thousands or even millions per cubic centimeter. Whenever there are considerable quantities of organic matter present in water, there is naturally a rapid multiplication of bacteria, providing temperature and other conditions are satisfactory.

Economic Importance of Impure Water

An impure water may be defined as one which contains disease-producing bacteria or which contains an excessive quantity of organic material susceptible to putrefaction or decay.

A number of diseases of man and animals are transmitted through drinking water. The most common of these

in man are typhoid fever, paratyphoid fever, Asiatic cholera and dysentery (both amebic and bacillary). In every case the organisms causing these diseases are forms that multiply in the intestines of those who are infected and can contaminate water only when the body excretions, that is, the feces and urine of those harboring the organisms, gain access. It is evident, therefore, that to a very great extent these diseases are transmitted only by water which contains excreta from those having the disease.

Diseases of animals are not so frequently transmitted through water. It is probable that occasionally animals, drinking from brooks or streams which have, farther up in their course, passed through farms where there are diseased animals, may contract such diseases as hog cholera or anthrax. Certain diseases of the alimentary tract in some respects closely resemble the paratyphoid fever of man and may also be transmitted in the same fashion.

The contamination of natural waters with considerable quantities of putrescible material, particularly sewage, is important also in other ways. The water of the streams containing a large percentage of sewage of factory wastes may become so polluted as to stop the development of the normal water fauna. It has been recognized, for example, that most of the so-called game fish will not live or develop in water containing any considerable admixture of sewage. Some fish can live in larger proportions, such as the carp. The presence of sewage also prevents the growth of insects and crustacea which constitute the food for these fish. It may also prevent the growth of the fresh water mussels or clams so important as a source of supply of material for manufacturing.

Large quantities of sewage or other organic material, such as factory wastes in the water of running streams, may also create a nuisance as a result of putrefaction. The presence of such organic matter in the water soon leads to the using

up of all of the dissolved oxygen, the organic matter then under these conditions undergoes putrefaction and malodorous gases may be thrown off.

Methods of Determining Purity of Water

The degree of desirable purity in water varies according to circumstances. A water which is to be used for drinking purposes must naturally have a much higher degree of purity than the water of some stream in which it is necessary only to prevent undesirable odors and putrefaction, that is, the development of a nuisance. The methods for determining whether or not water is suitable for drinking purposes and the methods for determining whether or not water is so heavily contaminated as to injure the water fauna such as fish and mussels or create a nuisance are quite different. They will be considered in order.

Methods of Determining the Potability of Water.—When methods of determining numbers of bacteria by means of plate counts were first introduced, it was assumed that this would give a reliable test as to the suitability of water for drinking purposes. In course of time, however, it developed that there is not always a direct relationship between the numbers of bacteria present and the potability of the water. The amount of food material required by microörganisms is very small indeed in some cases, how- water from a well, for example, which contains of the than 8 or 10 bacteria per cubic centimeter, wh there are to stand in a warm place for twenty-four ho of producing may have bacteria present by the thousands tose, quite cer- organisms may all be harmless and do not ing agents such it for drinking purposes. Although the amounts of water numbers is therefore not a reliable test. s containing either very helpful. In general it is true th hich does not induce not contain large numbers of bacte quantities as great as considerable amounts of organic r certainly free from

water containing large numbers of bacteria, therefore, is suspicious and should not be used until investigation has shown that the bacteria present are not derived from any source which might prove harmful or injurious.

Many attempts have been made to formulate a standard for potability of water, using the number of bacteria present per cubic centimeter as an index. These have in general proved impracticable. However, it may be said that water containing less than a hundred bacteria per cubic centimeter is usually, although not always, potable, and when bacteria are present in numbers greater than a thousand per cubic centimeter it usually indicates a water which is not suitable for drinking, certainly a water which should be investigated carefully before it is used.

Inasmuch as the bacteria which transmit disease through water are organisms present in the alimentary tract, it is evident that if organisms can be identified which are characteristic of sewage, that is, of human and animal feces, their presence in water will prove the presence of sewage in the water and the unsuitability of the water for drinking purposes, providing, of course, it can be shown that these same organisms do not exist in nature. A large amount of work has been done in recent years on this problem. in wateusly, if water upon examination is shown to contain Some fishular disease microörganism such as the typhoid, it The presences. It is evident, however, that any water conand crustacea minated by sewage will sooner or later contain may also prevenor that it at least is open to pollution from clams so importa typhoid patients. While not impossible, it manufacturing. isolate typhoid and similar bacteria from

Large quantities or are present because they are generally as factory wastes in thch smaller than other intestinal forms. create a nuisance as a reempts are therefore rarely made to of such organic matter irom infected water supplies. Atten-

tion is directed almost entirely to the recognition of organisms which are characteristic of sewage.

The organism usually sought to determine the potability of water is the *Bacterium coli,* and in some cases certain of its close relatives. *Bacterium coli* is a normal and constant inhabitant of the alimentary tract of man and of most, if not all species of warm-blooded animals. It is present in enormous numbers in the stools of man. While certain closely related forms such as *Bacterium aërogenes* may be found not infrequently in soil, *Bacterium coli* itself apparently does not live outside the human or animal body for any great period of time. Its presence in water accordingly is an indication of comparatively recent contamination with sewage. It has come, therefore, generally to be regarded as the most satisfactory indication of fecal contamination of water.

Many methods, both qualitative and quantitative, have been devised for determining the presence of *Bacterium coli* in water.

Presumptive Tests.—In bacteriological analysis of water to determine potability, it is customary first to make what is termed a *presumptive test.* This is a test which will readily differentiate waters which are quite certainly good from those which are suspicious. It is not customary, however, to condemn a water entirely upon the basis of the presumptive test. It is based upon the fact that there are not many species of bacteria which are capable of producing gas in fermentation tubes from the sugar lactose, quite certainly not in the presence of certain inhibiting agents such as ox bile. In making the test, varying amounts of water are added to a series of fermentation tubes containing either lactose bile or lactose broth. Water which does not induce gas production, even when used in quantities as great as one hundred centimeters, is quite certainly free from

sewage bacteria. If gas is produced in any fermentation tube it is highly probable that intestinal bacteria are present, although this does not constitute proof. It is customary to inoculate certain differential media from test tubes showing gas and by isolation of pure cultures from separated colonies identify *Bacterium coli.*

If *Bacterium coli* is shown to be present in quantities as small as ten cubic centimeters of water, it is evident that there has been more or less recent pollution by sewage. It is possible that in some cases confusion will arise due to the presence of organisms such as *Bacterium aërogenes* not uncommon in soils which simulate in many respects the characteristics of *Bacterium coli.* Condemnation of surface waters, particularly when only small numbers of gas-producing bacteria are present, should be made with caution.

It should be emphasized that water containing *Bacterium coli* is not condemned for drinking purposes because this organism is capable of causing disease when ingested, but because its presence indicates the presence also of sewage and opportunity for other microörganisms, such as the typhoid bacillus, to gain access to the supply.

Chemical Studies of Water to Determine Pollution.—Organic matter in sewage ordinarily will not become a nuisance, that is, give off disagreeable odors, providing there is sufficient aëration of the water in the stream. When large quantities of organic matter are present, the dissolved oxygen is quickly used up and changes which take place in the sewage, therefore, are necessarily anaërobic in nature. Anaërobic composition or putrefaction is apt to give rise to malodorous gases. The absence of oxygen, furthermore, prevents the growth of most forms of animal life in the streams. It is possible, therefore, by chemical analysis and comparison of organic matter found by such analysis with the amount of dissolved oxygen to determine something concerning the "putrescibility" of the water. In

many cases it is found necessary for cities not wholly to purify their sewage so that no disease-producing bacteria are present, but simply to purify it so that it will be no longer putrescible. Gross pollution of water may also be studied by chemical analysis for the determination of the amount of dissolved organic material (so-called albuminoid ammonia) the presence of nitrates and nitrites, and free ammonia.

Sanitary Qualities of Various Waters

As noted above, the sanitary quality of any water depends directly upon whether or not it is receiving sewage or other wastes which would make it harmful either to health of the user or to animal life contained.

Water in flowing streams is potable providing it does not receive sewage or factory waste. Most streams in the more thickly settled districts in America are polluted. There are some exceptions to this. Some of our larger cities use water from streams whose watersheds have been carefully protected and do not permit any sewage to gain access.

Lake water is in most cases somewhat less apt to be polluted than water in streams. Wherever it receives sewage, however, it is polluted.

Water from springs usually is of a high quality. Most springs receive water which has filtered through a considerable depth of soil and in some cases through rock. Some springs, however, particularly intermittent springs, are simply the opening of the channel of an underground stream which has direct communication with the surface of the earth. In limestone regions, for example, so-called sinks are found frequently. These are openings in the ground through which surface water passes. These communicate quite directly with these underground streams which, when they appear again upon the surface on the hillside, may contain all the contaminating materials present in the

streams which entered the sink. Such springs are apt to be muddy following rainy weather, showing the surface communication. Springs of this character, of course, are dangerous.

Water from cisterns carefully protected from dirt and adequately filtered is usually satisfactory for domestic use. There is very little opportunity in most cases for human excretions to reach such water.

Wells constitute the source of water supply for most rural communities and for farms. Studies which have been made upon the water in farm wells in different parts of the United States would seem to indicate that in general their character is relatively poor. In one survey made in Iowa, for example, about sixty per cent of samples secured from well water supplies were found to contain *Bacterium coli.* An examination of the surroundings of wells shown thus to be polluted will usually reveal the manner in which the contamination reaches the well. Many wells are located in barnyards and are not satisfactorily protected from surface water. It will be recalled that *Bacterium coli* is abundant in animal excretions as well as in human dejecta. The presence of such an organism in a farm water, therefore, does not necessarily mean that human excreta are finding their way into the supply, but that barnyard manure is gaining access. There is no reason to suppose that water containing diluted barnyard manure will produce disease in man unless it also contains human excretions. Any one who has had experience upon a farm will know, however, that water receiving drainage from the barnyard will contain not only organisms derived from animal sources but from human sources as well. In other words, while a water containing large numbers of *Bacterium coli* because of surface drainage from the barnyard, may be used for years without causing disease, nevertheless, inasmuch as it is subject to contamination, it is a source of constant danger.

Wells sunk to a sufficient depth and properly protected from surface wash practically always will yield pure water, provided there are no cracks or crevices in rock through which surface drainage may reach the water in the well. The latter do not frequently occur except in limestone regions. Wells in sand, particularly if clay has been penetrated in digging, usually will contain water of high sanitary quality. Care must be exercised, however, to see that privy vaults and cesspools are some distance removed. It is true that passing polluted water through sand or even gravel will rapidly remove the bacteria present by filtration; nevertheless constant passage of heavily contaminated or polluted water may in some cases cause microörganisms to cross considerable distance and eventually lead to pollution of wells.

Methods of Making Impure Water Potable

It is evident that the most satisfactory method of making and keeping water suitable for drinking purposes is entirely to prevent initial contamination. As has already been noted this sometimes is done for streams by protecting their watersheds, being sure that no sewage of any kind enters the stream. The same is true of lakes and other natural sources of supply. In some cases, however, it is necessary for cities and communities to make use of a supply of water, such as a stream or river, which receives sewage at some point farther up on its course. The water, therefore, is more or less contaminated and must be purified before it can be used. Purification is generally brought about in one of four ways: first, by the self-purification which is constantly going on in streams and lakes; second, by coagulation and sedimentation; third, by a chemical treatment; and fourth, by filtration.

Self Purification of Water.—It is a matter of common observation that streams, although they may be heavily

polluted at some portion of their course, will eventually apparently regain their purity at some distance down stream. The factors which have to do with such purification have been carefully studied in connection with several of the rivers and streams in this country. The most important of the factors which have to do with self-purification are as follows:

First, the *oxidation and removal of organic material* through the activity of the decay and putrefactive microorganisms. This occurs relatively rapidly, particularly if the water in the streams is sufficiently agitated to receive an abundant supply of oxygen.

Second, *antibiosis.* Microörganisms which develop most abundantly in the water of streams and sewage are more or less unfavorable for the life, certainly for the development, of the pathogenic bacteria such as the *Bacterium typhi.* As a result the latter organisms die off more or less rapidly. This is an example of what has been termed antibiosis, the antagonistic influence of one form of life upon another.

Third, *sedimentation.* Microörganisms are constantly falling to the bottom of the stream and remaining there, usually dying off rather rapidly. This sedimentation occurs particularly rapidly when there is more or less sediment in the stream which settles out. Bacteria show a decided tendency to cling to the surface of solid particles in suspension. As these settle out, the bacteria would of course pass down with them.

Fourth, the *antagonistic action of protozoa.* It is an easily demonstrated fact that many of the protozoa live upon bacteria. A sample of sewage, for example, allowed to stand for a few hours usually will be found to swarm with protozoa feeding upon the bacteria. These protozoa are quite abundant in the water of polluted streams and assist materially in its purification.

Fifth, *filtration.* The water in any stream constantly

encounters obstacles larger or smaller which produce a certain amount of filtering action. The water in a stream which is passing slowly through a wide bed of water plants or algæ is quite effectually filtered. Microörganisms tend to cling to the surface of the plant stems, sand, etc., with which they come in contact.

Sixth, *food and temperature conditions* are in general unfavorable to the growth of pathogenic bacteria and these die off relatively rapidly in consequence.

The distance which any water which has become contaminated must flow in order to become adequately purified differs with the conditions. Rapidity of flow, temperature, amount of dilution, and amount of contamination will all have their influence. In some cases evidence of sewage contamination will disappear within a few miles; under other conditions it will exist for some hundreds of miles down stream.

Coagulation and Sedimentation.—When certain chemicals such as iron salts, alum, etc., are added to waters containing sediment or organic material, the hydroxides which are more or less gelatinous in nature are generally formed. These cause coagulation or agglutination of the particles in suspension and lead to relatively rapid sedimentation. Most of the microörganisms are removed with the suspended material in this process. Usually, however, mere coagulation and sedimentation is not trusted to remove all harmful bacteria from polluted water, but it is an essential preliminary process in the purification of water of most streams used as a source of supply by cities.

Chemical Treatment.—In some cases where it has not been found practicable absolutely to eliminate harmful bacteria by other processes, the water supply of the city is treated with some chemical which will, on the one hand, destroy such harmful microörganisms, and on the other not injure the flavor of the water, prove poisonous, or injurious

in any way to consumers. Within recent years the most common chemicals to be employed have been bleaching powder, efficient because of its ability to release free chlorine, and free chlorine gas itself. These usually disappear relatively quickly by combining with various bases. As a result there is a temporary but efficient disinfecting action.

Filtration.—Many cities purify their water (usually after coagulation and sedimentation) by a process of filtration. It has been found by experience that if water is allowed to settle through a bed of sand, bacteria are quite largely eliminated. It must not be supposed that the openings between the sand grains are so small as mechanically to prevent the passage of the bacteria. Experience has shown, however, that when water is being filtered in this fashion, the grains of sand soon become coated with a gelatinous covering of microörganisms which oxidize the organic materials of the water with which they come in contact. These gelatin-coated sand grains have large powers of adsorption, and the microörganisms coming in contact with them usually cling to their surfaces. It is evident that sand filters become more efficient after a moderate amount of use. Eventually, of course, the surface coatings may be so heavy as to obstruct the water and it becomes necessary to clean them.

Efforts are sometimes made in the home to purify water by passing it through porcelain filters. When entirely free from cracks and freshly cleaned, these are relatively efficient, but like the sand grains, the pores in the filter are in most cases large enough eventually to permit the passage of microörganisms. They are, therefore, not usually advised for permanent installations for the purification of water.

Emergency purification of water can be quickly and easily effected by boiling. All of the disease-producing bacteria are destroyed at this temperature.

Sewage Disposal

In addition to the diseases already enumerated as transmitted through water contaminated by sewage, there are some other diseases transmitted quite directly from human excretions. The most important of these in the United States is the hookworm disease or uncinariasis. The hookworm lives during the latter part of its life history in the intestines of man. The eggs are passed out with the feces, hatched and gain access to the human body again only through the skin, usually the skin of the bare foot. It is evident, therefore, that proper disposal of the body excreta will stamp out this disease completely. The disease is primarily a rural disease in the south and has drawn attention to the necessity of better methods of disposing of the body excreta than are frequently used.

The rural and city problems of disposal of sewage are somewhat different. The presence of large numbers of people in a community necessitates the disposal of the sewage in order to prevent the creation of nuisances and to safeguard health. In practically all cases in America this is effected by the use of water. The farm and rural problem is complicated by the fact that expert advice as to disposal of sewage is usually not available. Fundamentally the reasons for proper sewage disposal are quite as potent in rural districts as in the city. Human excretions must be disposed of in such a manner that they will not come in contact with human beings or find their way into water or food.

The Rural Problem.—The necessity of disposing properly of human excretions of the farm can hardly be stressed too strongly. They must be so disposed of that they will not be a nuisance because of their odor; they must be so placed that they cannot come in contact, directly or indirectly with

human beings; they should as far as possible be protected from flies and vermin which might carry disease organisms present to the food of man; and particular care should be used not to allow them to gain access to drinking supply.

Probably in most rural communities the privy vault is most used for this purpose. Wherever there is any danger of contamination of wells, it should be water tight and arranged so that it is possible to clean at intervals. The addition of lime or bleaching powder in some cases will reduce odors. Proper covers should be provided so that flies cannot gain access and breed in the excreta.

The modern farm is rapidly becoming equipped with the same sanitary conveniences as the city home. In most cases this means the use of water-flushed closets and water-borne sewage. The installation of such necessitates the building of some adequate sewage disposal plant. In some cases, it is necessary simply to pass the sewage into a suitably located concrete cistern or septic tank in which the solid materials settle out and gradually decompose, finally for the most part dissolving in the water. The overflow from this septic tank may be treated in a variety of ways. In some cases it is found practicable to pass it through drainage tile laid in sandy soil, so that the liquid will rapidly seep away into the ground. In other cases filter beds, usually covered and constructed of sand, have been used, with tile laid below. As the effluent from the septic tank spreads out over the sand, it filters through, passes into the tiles below and is carried away to a convenient stream or emptied into some drainage tile. In some cases it is found expedient to install an additional chamber beyond the septic tank which will automatically flush at intervals or when filled to a certain height. Sewage disposal plans show a great variety of types. Directions for their construction and operation may be found in various government and experiment station bulletins. When properly constructed, they probably con-

stitute the ideal solution for the disposal of the sewage and waste from the country home.

The City Problem.—Rural communities are in some respects quite as much interested in the disposal of sewage from the cities as are the cities themselves. There is a general tendency wherever possible for cities to empty their unpurified sewage directly into streams or lakes or into the ocean. This pollution of natural waters, of course, interests those who live upon farms, through whose pastures the streams pass, or which border upon lake, bays, etc. which may be heavily polluted. It should be noted that in America at least, city sewage is practically universally water carried. In general it is diluted when it is emptied into streams. In some cases it is impounded in large reservoirs and there treated with certain chemicals, such as alum, or with acids which precipitate the solid materials as sludge. This latter is eventually dried and may be used for fertilizer. The supernatant liquid is allowed to pass without further treatment into streams. In some cases where sewage has been disinfected by the addition of chlorine or by the addition of acid, the microörganisms which are present are largely destroyed. Where the city is not so large as to make it impracticable, septic tanks and filter beds of various types have been employed. In recent years a modification of this has appeared in the use of the so-called activated sludge method. It has been found by experience that if sewage is drawn into a large tank and air bubbled through it, it can be rapidly purified. The more insoluble materials settle out in the form of a sludge and the clear supernatant liquid is allowed to discharge into a stream. Contact beds and sprinkling filters have also been used for purposes of purification.

CHAPTER XXXVIII

BACTERIOLOGY OF MILK

THE following table summarized from Van Slyke's analyses will demonstrate that milk is an exceedingly complex mixture of substances:

MILK	Water	87.1			
	Solids	12.9	Fat	4.0	
			Solids not fat	8.9	Proteins 3.3 (Albumen 0.7, Casein 2.6)
					Milk-sugar 4.9
					Ash (salts) 0.7
		100.		12.9	8.9 3.3

Some of the constituents probably are in true solution. Such, for example, are some of the salts and the milk sugar. Others, such as the proteins, are in colloidal solution, and the fat in the form of microscopic globules forms an emulsion. It will be noted that about 87 per cent of the milk is made up of water and 13 per cent solids, of which about 4 per cent should be fat and the remainder milk sugar, protein and ash.

Fermentative and Other Changes in Milk.—Common observation of the changes which occur in milk when it is allowed to stand after milking, show that there are several which follow each other in sequence. Ordinarily there are differentiated the stages of slight souring, curdling and eventual digestion of the curd. Careful laboratory studies show that the number of stages is really greater than those usually enumerated. They may be listed as follows:

1. Bactericidal stage.
2. Development of lactic acid.
3. Neutralization and fermentation of lactic acid.
4. Decomposition of the milk proteins.

In addition to the stages listed, milk frequently undergoes

other changes as well, such as sweet curdling, ropiness, soapiness and discoloration by pigment. The stages enumerated above will be discussed in order.

Germicidal Stage.—Usually milk contains some bacteria when drawn from the udder. Others rapidly find their way into it from the milking utensils, from dust, dirt, the milker, etc. Careful observation of the apparent numbers of bacteria present in this milk indicate that there is a period of diminution. The numbers of bacteria show a tendency to decrease rather than to increase. It has been assumed, therefore, that freshly drawn milk has a certain amount of germicidal power. Experimentally it may be readily demonstrated that freshly drawn milk, when inoculated with considerable numbers of many different kinds of bacteria, will show a decided diminution in the numbers upon plating. This action continues for a longer period when the milk is held at a low temperature and disappears more quickly at a higher temperature. There has been a considerable amount of argument in the scientific world as to whether this so-called germicidal action really represents the actual destruction of bacteria, or whether it simply means the bacteria have clumped together or agglutinated and upon plating the number of colonies is reduced, although the actual number of bacteria may even have increased. The evidence seems to indicate that there is some actual decrease in the numbers of bacteria. Various kinds of antibodies have been detected in milk, particularly bacteriolysins. Microscopic examination, furthermore, reveals considerable numbers of white corpuscles or leucocytes. These do not cease their activity for some time after the milk is drawn and it is possible that they may continue phagocytic action and destroy some microörganisms. This germicidal action never leads to the complete sterilization of the milk. It may be that the action is to a certain degree specific, some species of bacteria being destroyed while

others are not injured. This ability to destroy microörganisms is lost when milk is heated above 80° C. It should be emphasized that the germicidal action in milk, while of some assistance in holding down the number of bacteria, can never be depended upon to destroy the disease-producing germs which have gained access.

The Development of Lactic Acid.—The organisms responsible for the formation of lactic acid have been discussed in the chapter on lactic acid fermentation. In milk usually these bacteria develop rapidly, particularly if the milk is not kept chilled. Milk which contains 0.4 per cent of lactic acid has a decidedly sour taste and the milk curdles when the acidity reaches about 0.8 per cent.

Disappearance of the Acid.—Sour milk exposed to the air soon comes to have a coating or scum composed very largely of *Oöspora lactis* and other molds. These oxidize the lactic acid, that is, utilize it as food, forming carbon dioxide and water. Part of the acid also combines chemically with some of the proteins of the milk, particularly the caseinogen.

Decomposition of Protein.—Some of the lactic acid bacteria produce small quantities of proteolytic enzymes and lead to some digestion of the curd. This usually, however, is not apparent to the eye. Upon the neutralization or oxidation of the acid, putrefactive forms become active. Certain molds growing upon the surface of the milk produce proteolytic enzymes in considerable quantities. The proteins, particularly the caseinogen of the milk, are soon digested and transformed into peptones, polypeptids, amino acids, etc.

Abnormal Milk Fermentations

Usually when milk is allowed to stand, the lactic acid bacteria are present in sufficient numbers, so that their multiplication prevents the development of other forms of

bacteria. In some cases, however, particularly when there is unusually heavy inoculation of other forms, the latter may develop and the milk will not show normal souring. Milk, for example, which has been heated nearly to the boiling temperature, will contain no lactic acid bacteria. The spores of many of the putrefactive and decay-producing forms, however, will remain. These will develop when the milk is maintained at room temperature and lead to various changes such as sweet curdling. Many of the organisms belonging to the group of *Bacillus subtilis* bring about this change. The sweet curdling is due to the action of a rennetlike enzyme produced by the bacteria. In most cases the microörganisms proceed to decompose the curd, transforming it into amino acids and ammonia.

Under the discussion of capsule formation by microorganisms, it has already been noted that there are many species which are capable of producing sliminess or ropiness in milk. Some of these species are aërobic and produce the sliminess near the surface of the medium in contact with the air. Other forms apparently are facultative and produce sliminess throughout the medium.

Pigment-producing bacteria sometimes cause trouble in milk. Species are known which form blue islands on the surface of the milk, others change the milk itself to red, yellow or even brown or black. Other species have been described which produce undesirable flavors, in some cases developing bitter milk and soapy milk.

Bacterial Infection of Milk

Bacteria gain access to milk in a variety of ways. It is of considerable practical significance, therefore, to know of the sources of most importance. It is evident that some sources of bacterial contamination are important because of the numbers of bacteria which they will contribute, others are important rather because of the kinds.

Udder Infection.—It has previously been stated that the normal healthy tissues of animals usually do not contain living bacteria. This is true of the tissues of the mammary gland. However, there are apparently some bacteria which find conditions favorable for development in the milk cistern and in the larger ducts of the udder. The number found here differs greatly with different individuals, some animals normally having large numbers, others comparatively small numbers or even none. It is apparent, therefore, that the milk first drawn from the udder usually will contain somewhat larger numbers of bacteria than that drawn later. Very rarely do the numbers of bacteria amount to more than 100 per cubic centimeter in the freshly drawn milk. Animals revealing larger numbers usually are suffering from some udder infection.

Bacteria from Body Surfaces.—When animals are milked by hand into an open pail, it is inevitable that particles of dust and dirt adhering to the skin and hair will fall into the milk. Usually these are covered with large numbers of bacteria. If the animal is filthy, the numbers gaining access to the milk in this way may be considerable. Under usual conditions, however, the proportion of bacteria gaining access in this manner is not so large as from some other sources. It should be noted that this source of bacteria is very largely avoided in the use of the milking machine.

Bacteria from the Dust.—If the air in the stable or barn in which the cows are being milked is filled with dust, some will, of course, gain access to the milk and add to the numbers of bacteria present. Unless conditions are very bad, however, the total number of bacteria gaining access in this manner is not sufficient to be notable. This source of infection is also, in part at least, guarded against by properly constructed milking machines. The organisms gaining

access from this source are usually chromogenic cocci or spore-bearing bacilli.

Bacteria from the Hands of the Milker.—Undoubtedly bacteria may gain access to the milk from the hands of the milker. Bacteria from this source are particularly objectionable inasmuch as they are of human origin, and if the individual is suffering from any disease, there is a possibility of inoculating the milk with the organisms capable of causing this disease and thus spreading it through the milk. Many instances of infection in this manner are on record. Typhoid fever has been spread as a result of milkers being carriers of the disease. Diphtheria and scarlet fever have also been transmitted through milk.

Bacteria from Milking Vessels.—Studies made by several of the experiment stations in the United States have shown that this is probably the most important source of initial infection by bacteria. Unless all milk utensils are thoroughly sterilized there is a tendency to add many bacteria to the milk. Milking machines in particular are difficult to keep clean. The tubes should be thoroughly sterilized if gross contamination by bacteria is to be avoided. In the period elapsing between the time when the milk is drawn from the animal until it is finally delivered to the consumer, there may be numerous opportunities for contamination by bacteria.

The number of bacteria actually present in the milk at the time it is delivered to the consumer will vary according to the relative importance of several factors. In the first place, the number of bacteria gaining access to the milk during the process of milking will be important. Milk that is kept at a low temperature will allow but slow multiplication of microörganisms. Milk kept at a higher temperature or room temperature will show very rapid increase in bacteria present. The temperature, then, at which milk is held, has very important bearing upon the total number present

when delivered. It is evident also that the number of bacteria present will be a function of the time which has elapsed since the milk was drawn. It will also be affected by the care with which it has been handled and whether or not it has been pasteurized.

It should be recalled that milk is an excellent medium for the growth of many microörganisms, and if given the opportunity they will multiply until great numbers are present.

Disease Bacteria in Milk.—From the standpoint of the sanitarian, the fact that there are certain kinds of bacteria present in the milk may be more important than the numbers. Large numbers of lactic acid bacteria, for example, may be without particular significance from the standpoint of health, but the presence of any disease-producing organisms is apt to cause human infection. Evidently the most important of the diseases transmitted by milk are various diarrheas and dysenteries of young children. Whenever there has been a marked improvement in the character of the milk supply of our larger cities, there has been coincidently a marked decrease in the death rate, particularly in the morbidity rate among young children. Apparently the alimentary tract of the child is more susceptible to an infection of this kind than that of the adult. Particularly in summer when bacteria are apt to multiply to large numbers in milk there is trouble with so-called "summer complaint." The only safe procedure when milk does not come from a source which is entirely above suspicion is to pasteurize it.

Among the diseases attacking adults and transmitted by milk is typhoid fever. A number of epidemics of this disease has been traced to infected milk. It is usually not difficult to differentiate epidemics spread by milk from those which have their origin in contaminated water by a study of the distribution of the cases and by the fact that in most such epidemics a considerable proportion of the victims are

children. In some instances it has been found possible by marking the location of infected families on a map to show the milk route of some individual. Inasmuch as typhoid fever is not a disease of cattle it is apparent that the organisms can gain access to milk only after milk has been drawn, through careless handling, use of contaminated water, or perhaps more commonly by contact with a typhoid carrier or one who is just coming down with the disease or has recently recovered from it. Scarlet fever and diphtheria have apparently in some cases been transmitted through milk. Inasmuch as these no more than typhoid are diseases of animals, it is apparent that the organisms have gained access only through careless handling.

The more important of the organisms which may be present in market milk is the *Bacillus tuberculosis* which has already been discussed, as has also the relationship of tuberculosis in animals to the disease in man. It seems to be a well-demonstrated fact that the use of milk from tuberculous animals causes a considerable percentage of the cases of tuberculosis, particularly of the scrofulous type, in children. Milk used for human consumption should, if possible, be secured from animals that have been proved to be free from disease. If this is impossible then pasteurization should be resorted to.

Other diseases which have been occasionally transmitted by the milk of the cow are foot and mouth disease, milk sickness, Malta fever and anthrax. These diseases are very rare and occur only occasionally in animals in the United States. They are an unimportant type of disease in man.

Numerous instances in recent years have been recorded in the United States of cases of so-called septic sore throat, caused by a species of the genus *Streptococcus*. It is probable that in some cases the organism has entered the milk from human carriers, but in other cases it is possible that it

has been transmitted from animals affected with inflammation of the udder (mastitis).

Classification of Market Milk

Methods of classifying market milk differ with the locality. In a number of cities milk has been divided into four grades: certified milk, inspected milk, pasteurized milk, and uninspected milk.

Certified Milk.—The term certified milk was introduced originally to designate milk produced by a dairy which had a regular system of inspection by some board of health or committee of physicians. The requirements which must be met when producing milk of this type are numerous and must be rigidly adhered to. Among the more important items are that the animals must be shown by continual veterinary supervision to be free from contagious diseases. The attendants, particularly the milkers, must be in good health; the stables must be sanitary, well lighted, free from dust; milking vessels must be sterile and every reasonable precaution used to prevent bacteria getting into the milk. When milk has been drawn from the animal, it must be quickly cooled, sealed in bottles and kept cool until it has been delivered. Furthermore, in most cases a bacterial standard is imposed and certification is withdrawn if milk is found consistently to have more than ten thousand bacteria to the cubic centimeter. In a few instances milk can be certified when the numbers do not reach more than twenty-five thousand. Additional requirements over those of the other grades of milk make it necessary to charge a higher price for certified than for other types of milk.

Inspected Milk.—Inspected milk is milk that comes from a dairy where the cows have been inspected and certified to be free from tuberculosis. It must be drawn and cared for under sanitary conditions, but the extreme precautions used in certified milk are not required.

Uninspected Milk.—Even yet in the United States most milk which is sold to the consumer is uninspected and no sanitary control whatever is maintained. In a few instances, cities have established maximum standards for bacterial count, and withdraw licenses from those who sell milk containing more than the maximum number of organisms. Such ordinances, however, are difficult to enforce.

Pasteurized Milk.—Pasteurization has already been defined as heating milk for such a time and temperature as will destroy any disease-producing bacteria present and not seriously injure the flavor or creaming qualities of the milk. In some cases, the term "pasteurized" has been used to indicate a milk of supposedly superior quality that has not been actually heated. This, of course, is misbranding. Pasteurization is a technical operation, requiring some intelligence on the part of those carrying out the process. In some instances the label "Pasteurized" has been used to mean that milk has passed through an apparatus called a "pasteurizer" whose efficiency is wholly unknown.

Pasteurization may be carried out in the home. It is best then to heat the milk in stoppered bottles to 60° c., hold at this temperature for at least 20 minutes and cool rapidly. In commercial pasteurization, two types of pasteurizers are used. In the so-called flash process, the milk is heated to the required temperature, usually 80° to 85° c., and maintained at that temperature for from 30 seconds to a minute. It is then cooled and maintained at a low temperature until distributed. In the holder process, the milk is kept at a temperature required, 60° to 65° c., for about half an hour. A combination of the two methods is particularly efficient. Usually over 99 per cent of the bacteria present in milk can be destroyed by proper pasteurization. All pathogenic microörganisms should be destroyed.

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